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Wenyu Lin, *University of San Francisco*
Michael A Rice, *University of Rhode Island*
Paul K Chien, *University of San Francisco*

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THE EFFECTS OF COPPER, CADMIUM AND ZINC ON PARTICLE FILTRATION AND UPTAKE OF GLYCINE IN THE PACIFIC OYSTER *CRASSOSTREA GIGAS**

WENYU LIN,† MICHAEL A. RICE‡ and PAUL K. CHIEN§

†Department of Fisheries Animal and Veterinary Science, The University of Rhode Island, Kingston, RI 02881, U.S.A.; and §Department of Biology, University of San Francisco, San Francisco, CA 94117, U.S.A.

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Abstract—1. The filtration rate (volume of water completely cleared of collodial carbon per unit time) by control oysters is 36.60 ml/g hr ± 7.68 (sd).

2. Filtration rates decrease with increasing concentrations of Cd^{2+} and Zn^{2+}.

3. In 8–16 mg/l Cu^{2+}, filtration rates are significantly higher than the control, but in Cu^{2+} concentrations above 32 mg/l, filtration rates are lower than controls.

4. Influx of ^{14}C-glycine is characterized by Michaelis–Menten kinetics with J_{max} and K_t values of 1.85 ± 0.097 μmol/g hr and 33.7 ± 4.6 μM respectively.

5. The uptake rate of glycine from 1 μM solution is 37.79 μmol/g hr.

6. In order of degree of inhibition of glycine uptake, $Cu^{2+} > Cd^{2+} > Zn^{2+}$.

7. In 128 mg/l Cu^{2+}, glycine uptake rate is reduced to 3.96 nmol/g hr or 10.5% of control.

8. The rate of glycine uptake by filter feeding bivalves is dependent on rate of water pumping rate.

9. The volume specific glycine transport (amount of glycine transported/unit volume of seawater completely cleared of colloidal carbon) by control oysters in 1 μM glycine concentrations is 1.03 μmol/l.

10. The volume specific glycine transport remains constant in increasing Zn^{2+} concentrations, and declines in increasing Cu^{2+} concentrations, suggesting differential effects of the metals on particle filtration and the epithelial amino acid carriers.

11. The apparent volume specific glycine transport increases to 2.14 μmol/l in 128 mg/l Cd^{2+}. This volume specific transport greater than the glycine concentration in the medium suggests that there may be uptake of cadmium complexed glycine by the oysters.

INTRODUCTION

The uptake and accumulation of heavy metals by oysters has been studied over the past hundred years. Lankester (1886) noted that a green coloration in some oysters was due to copper accumulation. Studies begun in the 1920s and 1930s in efforts to protect public health concluded that increased heavy metal content of oyster tissues was due to industrial pollution and thus was also an explanation for declining oyster production which had peaked in the United States in 1908 (Hunter and Harrison, 1928; Coulson *et al.*, 1932; Galtsoff *et al.*, 1947). Some work has focussed on determining the acute toxicity of certain metals to oysters (e.g. Calabrese *et al.*, 1973), but there has been increasing awareness of the sublethal toxic effects of metals on various invertebrates including oysters (reviewed by Cunningham, 1979). Sublethal effects of heavy metals include decreases in oxygen consumption by various tissues, ultrastructural changes in epithelial and other tissues, and induction of specific metal-binding proteins (Engel and Fowler, 1979).

Few studies have been undertaken which have investigated the effects of metals on the rates of water pumping or nutrient acquisition by bivalves. Water flow through bivalve gills allows for feeding, respiration, and the removal of metabolic products, and can be potentially disrupted in the presence of metallic pollutants (Schulte, 1975; Mohlenberg and Riisgard, 1979; Palmer, 1980; Gerdes, 1983). In this

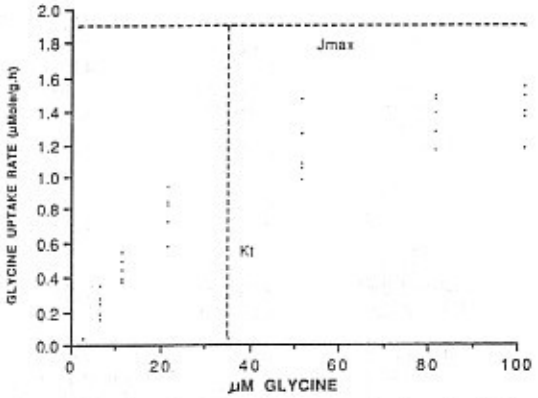

Fig. 1. Uptake of glycine by oysters is describable by Michaelis–Menten saturation kinetics. Individual points correspond to individual rate determinations. Kinetic parameters are J_{max} = 1.85 μmol/g hr ± 0.097 (sd) and K_t = 33.68 μM ± 4.61 (sd).

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‡Person to whom reprint requests should be directed.

study, one focus is on the effects of various metals on the rate of particle filtration by oysters.

In addition to filtration and assimilation of particulates, uptake of dissolved organic material directly from the environment has been known as a potential nutrition source for oysters (see reviews by Wright, 1982; Stephens, 1988; Wright and Manahan, 1989). Uptake of sugars and other organic materials from seawater by adult oysters has been known since the 1920s (Ranson, 1927; Yonge, 1928). However, the introduction of a technique for inducing steady pumping rates in oysters by artificially irrigating the mantle cavity has only recently allowed for the determination of instantaneous uptake rates of dissolved organic materials in environmentally realistic concentrations, as well as the reliable estimation of Michaelis–Menten saturation kinetics parameters (Rice and Stephens, 1987). Using this technique, we investigate the effects of copper, cadmium and zinc on the uptake of glycine and its subsequent distribution in sub-epithelial tissues of Pacific oysters.

MATERIALS AND METHODS

Pacific oysters were purchased from a commerical aquaculture facility in Drake's Bay, Marin County, California.

Table 1. The effect of Cu^{2+}, Cd^{2+}, Zn^{2+} on uptake and distribution of exogenous glycine in pacific oyster (uptake rate: nmole/g · hr). The initial concentration of glycine in the media was 1.0 μM.

Whole animal treatment	*P*	Mean (nmole/g · hr)	Sta. dev.	Min.	Max.	*n*
Control		37.793	6.456	31.25	46.09	6
2 mg/l Cu^{2+}	0.0008***	23.228	3.970	18.07	28.40	6
4 mg/l Cu^{2+}	0.0001***	20.243	2.038	17.73	22.73	6
8 mg/l Cu^{2+}	0.0000***	15.693	3.348	10.37	20.66	6
16 mg/l Cu^{2+}	0.0000***	15.777	2.765	11.94	19.35	6
32 mg/l Cu^{2+}	0.0000***	15.547	3.106	10.50	19.50	6
64 mg/l Cu^{2+}	0.0000***	8.544	1.479	6.79	10.10	6
128 mg/l Cu^{2+}	0.0000***	3.962	0.771	3.12	5.123	6
2 mg/l Cd^{2+}	0.0256*	27.972	5.563	20.17	34.76	5
4 mg/l Cd^{2+}	0.0035**	25.302	3.164	20.67	29.32	5
8 mg/l Cd^{2+}	0.0013**	22.776	3.700	18.97	28.74	5
16 mg/l Cd^{2+}	0.0003***	19.746	2.762	15.97	22.87	5
32 mg/l Cd^{2+}	0.0002***	19.518	2.482	16.79	23.08	5
64 mg/l Cd^{2+}	0.0001***	15.882	2.671	12.72	19.64	5
128 mg/l Cd^{2+}	0.0000***	10.454	2.065	7.61	12.97	5
2 mg/l Zn^{2+}	0.4606	35.242	3.890	30.70	40.14	5
4 mg/l Zn^{2+}	0.0700	31.346	2.881	27.90	34.54	5
8 mg/l Zn^{2+}	0.0195*	29.000	2.621	25.93	32.88	5
16 mg/l Zn^{2+}	0.0315*	29.330	3.973	23.80	33.47	5
32 mg/l Zn^{2+}	0.0048**	25.694	3.618	20.58	30.30	5
64 mg/l Zn^{2+}	0.0001***	17.328	2.952	14.31	20.94	5
128 mg/l Zn^{2+}	0.0000***	13.562	2.414	10.09	16.37	5

Muscle treatment	*P*	Mean (nmole/g · hr)	Sta. dev.	Min.	Max.	*n*
Control		5.183	0.936	4.195	6.623	6
2 mg/l Cu^{2+}	0.1684	4.437	0.655	3.705	5.108	5
4 mg/l Cu^{2+}	0.0367*	3.934	0.705	3.017	4.682	5
8 mg/l Cu^{2+}	0.0005***	2.870	0.531	2.299	3.624	5
16 mg/l Cu^{2+}	0.0004***	2.777	0.368	2.284	3.078	5
32 mg/l Cu^{2+}	0.0003***	2.491	0.506	1.796	2.942	5
64 mg/l Cu^{2+}	0.0000***	1.718	0.246	1.440	2.010	5
128 mg/l Cu^{2+}	0.0000***	0.542	0.106	0.398	0.663	5
2 mg/l Cd^{2+}	0.4874	4.791	0.838	3.980	5.970	5
4 mg/l Cd^{2+}	0.2097	4.562	0.449	3.853	5.010	5
8 mg/l Cd^{2+}	0.1477	4.335	0.815	3.321	5.530	5
16 mg/l Cd^{2+}	0.0063**	3.509	0.525	2.930	4.021	5
32 mg/l Cd^{2+}	0.0013**	3.029	0.498	2.470	3.573	5
64 mg/l Cd^{2+}	0.0005***	2.790	0.422	2.176	3.242	5
128 mg/l Cd^{2+}	0.0000***	1.894	0.309	1.508	2.340	5
2 mg/l Zn^{2+}	0.6774	4.961	0.743	4.125	5.890	5
4 mg/l Zn^{2+}	0.9361	5.140	0.813	3.990	6.020	5
8 mg/l Zn^{2+}	0.1598	4.364	0.809	3.473	5.637	5
16 mg/l Zn^{2+}	0.1150	4.302	0.685	3.671	5.428	5
32 mg/l Zn^{2+}	0.0051**	3.345	0.665	2.650	4.081	5
64 mg/l Zn^{2+}	0.0040**	3.259	0.672	2.451	4.173	5
128 mg/l Zn^{2+}	0.0001***	2.078	0.251	1.681	2.350	5

Hemolymph treatment	*P*	Mean (nmole/g · hr)	Sta. dev.	Min.	Max.	*n*
Control		2.071	0.293	1.776	2.403	6
2 mg/l Cu^{2+}	0.5994	1.864	0.192	1.605	2.107	5
4 mg/l Cu^{2+}	0.0743	1.595	0.258	1.173	1.784	5

continued

Table 1—*continued*

Hemolymph treatment	*P*	Mean (nmole/g · hr)	Sta. dev.	Min.	Max.	*n*
8 mg/l Cu^{2+}	0.0279*	1.491	0.259	1.174	1.817	5
16 mg/l Cu^{2+}	0.0376*	1.476	0.362	1.085	1.931	5
32 mg/l Cu^{2+}	0.0252*	1.481	0.257	1.223	1.859	5
64 mg/l Cu^{2+}	0.0051**	1.366	0.150	1.150	1.511	5
128 mg/l Cu^{2+}	0.0007***	1.129	0.209	0.904	1.345	5
2 mg/l Cd^{2+}	0.2527	1.769	0.233	1.465	2.068	5
4 mg/l Cd^{2+}	0.1168	1.685	0.179	1.462	1.928	5
8 mg/l Cd^{2+}	0.1131	1.678	0.191	1.467	1.972	5
16 mg/l Cd^{2+}	0.0580	1.603	0.195	1.398	1.904	5
32 mg/l Cd^{2+}	0.0182*	1.487	0.170	1.268	1.718	5
64 mg/l Cd^{2+}	0.0198*	1.483	0.209	1.245	1.783	5
128 mg/l Cd^{2+}	0.0026**	1.276	0.187	0.992	1.458	5
2 mg/l Zn^{2+}	0.3436	1.816	0.198	1.568	2.043	5
4 mg/l Zn^{2+}	0.2042	1.752	0.172	1.549	1.948	5
8 mg/l Zn^{2+}	0.1191	1.686	0.182	1.453	1.920	5
16 mg/l Zn^{2+}	0.0879	1.652	0.183	1.431	1.919	5
32 mg/l Zn^{2+}	0.0403*	1.557	0.217	1.329	1.873	5
64 mg/l Zn^{2+}	0.0057**	1.371	0.156	1.179	1.589	5
128 mg/l Zn^{2+}	0.0010***	1.145	0.225	0.894	1.415	5

Gill treatment	*P*	Mean (nmole/g · hr)	Sta. dev.	Min.	Max.	*n*
Control		139.222	26.356	108.60	173.60	6
2 mg/l Cu^{2+}	0.0106*	96.548	14.595	82.85	121.10	5
4 mg/l Cu^{2+}	0.0004***	70.824	10.065	59.28	84.31	5
8 mg/l Cu^{2+}	0.0000***	38.714	7.217	30.99	46.90	5
16 mg/l Cu^{2+}	0.0000***	40.936	5.554	35.89	49.70	5
32 mg/l Cu^{2+}	0.0000***	65.942	14.354	50.76	86.10	5
64 mg/l Cu^{2+}	0.0000***	37.729	7.232	28.525	48.60	5
128 mg/l Cu^{2+}	0.0000***	7.339	1.590	5.190	9.346	5
2 mg/l Cd^{2+}	0.6769	133.18	18.433	105.60	153.50	5
4 mg/l Cd^{2+}	0.5458	130.204	20.373	98.34	148.20	5
8 mg/l Cd^{2+}	0.0986	114.964	14.002	97.02	130.60	5
16 mg/l Cd^{2+}	0.0137*	97.476	16.626	79.90	119.30	5
32 mg/l Cd^{2+}	0.0039**	86.836	16.334	70.72	109.30	5
64 mg/l Cd^{2+}	0.0018**	83.434	11.170	69.70	96.50	5
128 mg/l Cd^{2+}	0.0001***	62.448	6.996	52.71	70.64	5
2 mg/l Zn^{2+}	0.9801	142.858	12.753	126.75	160.30	5
4 mg/l Zn^{2+}	0.3726	126.012	14.539	109.80	145.87	5
8 mg/l Zn^{2+}	0.0773	110.624	11.578	97.68	124.53	5
16 mg/l Zn^{2+}	0.1788	120.160	13.412	103.80	135.78	5
32 mg/l Zn^{2+}	0.0946	113.236	17.846	93.45	140.60	5
64 mg/l Zn^{2+}	0.0433*	106.562	17.793	85.83	133.10	5
128 mg/l Zn^{2+}	0.0000***	43.686	9.418	33.87	59.08	5

Mantle treatment	*P*	Mean (nmole/g · hr)	Sta. dev.	Min.	Max.	*n*
Control		17.290	2.292	14.72	21.14	6
2 mg/l Cu^{2+}	0.8959	17.072	3.095	12.12	19.97	5
4 mg/l Cu^{2+}	0.1126	14.714	2.571	10.49	17.10	5
8 mg/l Cu^{2+}	0.0028**	9.784	1.469	7.70	11.66	5
16 mg/l Cu^{2+}	0.0258*	13.593	2.726	10.30	17.46	5
32 mg/l Cu^{2+}	0.0465*	14.683	2.319	12.82	17.93	5
64 mg/l Cu^{2+}	0.0396*	14.571	2.885	11.80	18.13	5
128 mg/l Cu^{2+}	0.0000***	4.420	0.983	3.23	5.49	5
2 mg/l Cd^{2+}	0.3860	15.826	3.046	12.03	20.20	5
4 mg/l Cd^{2+}	0.1599	15.390	1.645	13.44	17.78	5
8 mg/l Cd^{2+}	0.1823	15.190	2.528	12.00	18.41	5
16 mg/l Cd^{2+}	0.0510	14.742	1.140	13.00	16.08	5
32 mg/l Cd^{2+}	0.0033**	12.604	1.415	10.94	14.16	5
64 mg/l Cd^{2+}	0.0008***	11.282	1.577	9.21	13.20	5
128 mg/l Cd^{2+}	0.0000***	8.058	0.957	6.91	9.32	5
2 mg/l Zn^{2+}	0.2979	16.022	1.233	14.89	17.91	5
4 mg/l Zn^{2+}	0.8507	17.020	2.315	13.56	19.48	5
8 mg/l Zn^{2+}	0.0401*	14.126	2.032	12.42	17.45	5
16 mg/l Zn^{2+}	0.3101	15.807	2.256	13.21	19.05	5
32 mg/l Zn^{2+}	0.0908	14.970	1.625	13.56	17.45	5
64 mg/l Zn^{2+}	0.0077**	12.777	2.043	10.97	15.97	5
128 mg/l Zn^{2+}	0.0000***	7.720	1.353	5.91	9.17	5

P is the probability of variation (ANOVA): if it is in the range of 0.05 to 0.01, the source of variation is considered statistically significant and is denoted by making the *P* value with a single star. *P* in the range of 0.01 to 0.001 is considered very significant and is denoted with two stars. When *P* is less than 0.001, it is considered highly significant and is denoted with 3 stars.

The oyster shell-free wet weight ranged from 8.65 to 24.89 g, and they were about 18 months of age. The animals were maintained unfed in natural seawater aquaria at 15°C. Oysters were acclimated to room temperature (22°C) prior to use in experiment. Experimental observations were carried out in artificial seawater (ASW) prepared according to Cavanaugh (1956).

The pumping of water through the oyster mantle cavity is easily disturbed, variable in rate, and unpredictable in onset. Steady water transport rates were produced by the procedures of Rice and Stephens (1987). A hole, 1 cm in diameter was bored at the incurrent edge of the shell. The mantle chamber was irrigated at 2.5 l/hr using a peristaltic pump to recirculate the 250 ml seawater in the experimental vessel. Steady water flow through the animal was indicated by the sustained decrease of glycine from solution. By use of an 8-channel pump, 8 experimental animals were able to be studied simultaneously.

The rate of glycine uptake was determined using mixture of ^{14}C labelled glycine at 320 Bq/ml (10 nCi/ml) and ^{12}C-glycine to produce total concentrations ranging from 1 to 100 μM. The effect of heavy metals on glycine uptake were determined using mixtures of 1 μM gly with various concentrations of Cu^{2+}, Cd^{2+}, and Zn^{2+}.

Samples (0.3 ml) were taken periodically from the medium and mixed with 3 ml liquid scintillation cocktail (Scintisol, Isolab). Influx of ^{14}C glycine was inferred by monitoring the depletion of radioactivity using a Searle Analytic 6892 Liquid Scintillation Counter (LSC) with corrections for background and quenching. At the end of 60 min, samples of tissues (gill, mantle and muscle tissue) were removed and weighed. Hemolymph was drawn from the pericardium and its total volume measured. Tissues were ethanol extracted for 24 hr and radioactivity measured. The data were fit to the Michaelis–Menten equation, using a non-linear least squares regression computer program (Duggleby, 1981).

The oyster filtration was studied by adding heavy metals to vessels containing oysters. "Aquadag" colloidal carbon was subsequently added. A similar vessel without oysters was run as a control for physical settling of the carbon. Water samples were taken for absorbance readings every 5 min using a Bausch and Lomb Spectronic 21 spectrophotometer. Following the filtration experiment, animals were sacrificed and the soft tissues weighed. Data corrected for physical settling of carbon were used to calculate the rate of water filtration from the formula of Jorgensen (1943):

$$m = \frac{M(\mathrm{Log}_e(C_0 - C_t))}{Wt}$$

where:

C_0 = initial concentration at time zero
C_t = concentration at time t
M = volume of water in the vessel in ml
m = filtration rate (ml/g · hr)
W = wet weight of soft tissue of animals in g
t = time in hr.

Alternatively, filtration rates were estimated from depletion rate constants derived from semi-logarithmic plots of colloidal carbon absorbance versus time.

To determine the variance between the control and different treatments using heavy metals, all data from each treatment were analyzed by using Descriptive Statistics and Analysis of Variance (ANOVA) to compare the significance of the differences.

RESULTS

Influx of ^{14}C-labeled glycine is well described by Michaelis–Menten kinetics (Fig. 1). The values for the maximum uptake rate of glycine (J_{max}) by the intact animal is 1.85 μmole/g hr $\pm$ 0.097 (sd) and the transport affinity constant (K_t) from the whole animal is 33.68 μM $\pm$ 4.61 (sd). Highest rates of glycine uptake were found in the gill. The J_{max} was 6.82 μmole/g · hr $\pm$ 0.423, suggesting a higher than average carrier density and that the gill is the primary site of glycine uptake. However, there was significant uptake of glycine via the mantle tissues.

Data concerning the effect of heavy metals on glycine uptake rate in Pacific oyster are presented in Table 1. At higher heavy metal concentrations, the rate of glycine uptake was lower. In the intact control animals, the glycine uptake rate from 1 μM solution was 37.79 nmol/g hr; whereas in the presence of 128 mg/l Cu^{2+}, it decreased to 3.96 nmol/g hr. Based upon the degree of inhibition of the glycine uptake rate, Cu^{2+} is the most toxic, followed by Cd^{2+} and Zn^{2+}. Highly significant reductions in glycine transport are elicited by 16 mg/l Cd^{2+} and 64 mg/l Zn^{2+}, but only 2 mg/l Cu^{2+} was necessary to cause a highly significant reduction in glycine transport.

In addition to reducing the rate of glycine transport in the intact animal, the divalent metals reduce the glycine transport rate in isolated epithelial tissues (Table 1). The uptake rate of the gill in the control is 139.22 nmol/g hr, and the rate decreases with the increasing Cu^{2+} concentration. In 128 mg/l Cu^{2+}, the uptake rate is 7.34 nmole/g hr, which is 5.3% of the control. As in the intact animal, Cd^{2+} and Zn^{2+} appear to be less toxic. In 128 mg/l Cd^{2+}, the uptake rate of glycine by the gill is 62.45 nmole/g hr, which is 44.85% of the control, and in 128 mg/l Zn^{2+}, the uptake rate is 43.69 nmol/g hr, or 31.38% of the control rate. Glycine is also known to be taken up from seawater by the mantle epithelia. Copper, cadmium and zinc similarly inhibit the rate of glycine uptake by this tissue.

Glycine taken up via epithelial tissues is subsequently transported to deeper tissues such as the hemolymph and the adductor muscle (Table 1). Copper, zinc and cadmium reduce the rate at which glycine appears in these tissues. This reduction may be the result of a direct affect of the metals on the mechanism of transepithelial transport, but more likely, it is a secondary effect of lowered glycine availability in the epithelial tissues.

The effects of divalent heavy metals on filtration rates appear to be more complex than the simple reductions of glycine transport with increasing metal concentration (Fig. 2). The mean particle filtration

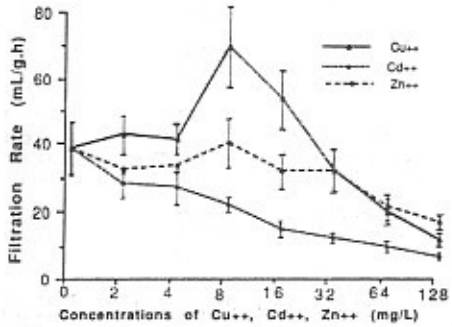

Fig. 2. Filtration rate or the volume of water cleared of colloidal carbon per hour is expressed as a function of increasing metal concentration. Data points are the mean of five determinations and error bars are one standard deviation.

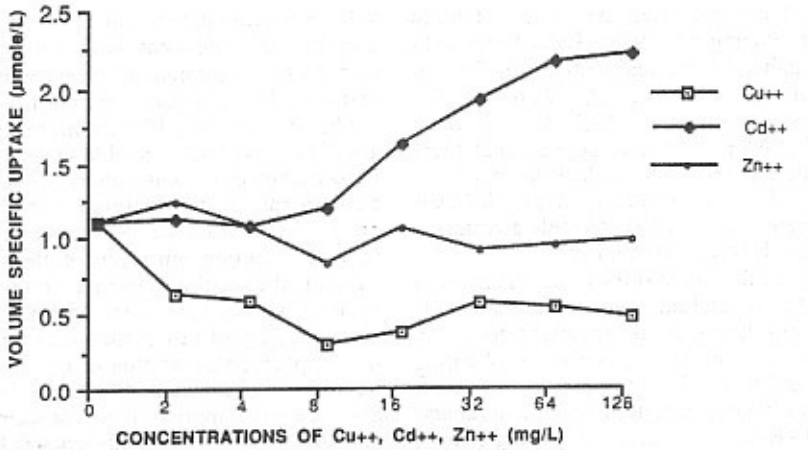

Fig. 3. Volume specific glycine influx rate is expressed as a function of increasing metal concentration. The volume specific rates were calculated from carbon clearance data and glycine uptake rates from 1.0 μM concentrations.

rate for control oyster oysters was 36.60 ml/g hr. ANOVA analysis of the data shows that the filtration rate in 8 mg/l and 16 mg/l Cu^{2+} are significantly higher than the control. Filtration rates are significantly lower than the control in 64 and 128 mg/l Cu^{2+}. The filtration rates decreased with increasing concentrations of Cd^{2+}. In Cd^{2+} concentrations higher than 8 mg/l, the filtration rates are significantly lower than the control ($P < 0.001$). In 128 mg/l Cd^{2+}, the filtration rate was 4.86 ml/g hr, which is only 13.3% of the control. Low concentrations of Zn^{2+} had little effect on the filtration rate, but at higher concentrations, the rate rapidly decreased to 5.58 ml/g hr (or 15.2% of the control) in 128 mg/l Zn^{2+}.

The amount of amino acids transported by filter feeding invertebrates can be expressed as a function of the amount of water transported by the animal. Although particle filtration is not strictly equal to the total water transport, it has been used as an index of water transport by filter feeding bivalves. The volume specific transport of glycine is expressed as μmoles of substrate transported per liter of water cleared of colloidal carbon (Fig. 3). The volume specific glycine transport by control animals (no added metals) in 1 μM gly is 1.03 μmol/l. The volume specific glycine transport decreases as a function of increasing copper concentration to a minimum of 0.40 μmol/l at 128 mg/l Cu^{2+}. Zinc does not significantly affect the volume specific glycine transport. Cadmium, in contrast, acts to increase the apparent volume specific glycine transport to a maximum of 2.14 μmol/l at 128 mg/l Cd^{2+}.

DISCUSSION

The uptake of glycine by the Pacific oyster closely follows Michaelis–Menten saturation kinetics (Fig. 1). The reported value of the Michaelis constant (K_t) of 33.7 μM and the maximal rate of glycine influx (J_{max}) of 1.85 μmol/g hr closely correspond to the respective alanine transport parameters of 33.7 μM and 1.79 μmol/g hr by *C. gigas* (Rice and Stephens, 1987). It is known from competitive inhibition studies that α-amino acid transporters in *C. gigas* have broad specificities (Rice and Stephens, 1987), so it is reasonable to assume that these two chemically similar amino acids will have very similar Michaelis–Menten transport characteristics.

The value of the Michaelis constant has been of considerable interest. Theoretical considerations have argued that there are natural selection pressures which result in enzyme systems and transport carriers with K values which fall within an order of magnitude of normally anticipated substrate concentrations (Atkinson, 1969; Crowley, 1975). Reported K_t value for some mussels are 1.7 μM for the blue mussel, *Mytilus edulis* (Jorgensen, 1976); 1.3–3.0 μM for the California mussel, *Mytilus californiaus* and 2.1–3.8 μM for ribbed mussel, *Modiolus demissus* (Wright and Stephens, 1978). Total free amino acids (FAA) in waters along the open ocean coastal habitat of California mussels is reported to range from 0.8 to 1.6 μM (Stephens and Manahan, 1984; Almeida *et al.*, 1989). Other studies of water column FAA concentrations in near shore coastal waters and embayments which are the habitat of *Mytilus edulis* and *Modiolus demissus* show ranges between 0.63 and 2.92 μM (Braven *et al.*, 1984). These data suggested that there is a reasonable match between expected environmental FAA concentration and the K_t values for at least the mytilids. However, there is a growing body of evidence that K_t values for oysters are generally higher than the mytilids. The current study and that of Rice and Stephens (1987) suggest that K_t values for alanine and glycine are in the 33 μM range for intact, activly pumping *C. gigas*. The only other study to date which investigated the kinetics of amino acid uptake by a post-metamorphic oyster is that of Rice *et al.* (1980), who reported that the K_t values for alanine and glycine influx into 1.5 mm *Ostrea edulis* juveniles were 16.8 μM and 12.55 μM respectively.

The relatively high K_t values may be consistent with the specific microhabitat of most oyster species which tend settle and attach to rocks or other oyster shells on the estuarine bottom, close to the sediment–water column interface. Sediment pore water may be one to two orders of magnitude higher in FAA

concentration than the overlying water column (Henrichs and Farrington, 1979). Indeed the sediments are postualted to be a major source of FAA in the water column (Jorgensen *et al.*, 1980). The few studies which have investigated FAA content of waters near the sediment surface suggest that there may be enrichment in this boundary layer (Davis *et al.*, 1985). Thus the relatively high Michaelis constants of oysters may be a reasonable adaptation for the estuarine benthic environment.

A number of studies have shown that the rates of FAA uptake by invertebrates are inhibited by the presence of various heavy metals. Invertebrates which have been studied include the polychaete, *Neanthes virens* (Rice and Chien, 1978); the polychaete *Nereis diversicolor* and the oligochaete, *Enchytraeus albidus* (Siebers and Ehlers, 1979); and the grazing marine gastropod, *Tegula funebralis* (Ueda, 1991). In addition, amino acid transport by isolated gill tissue of the Mediterranean mussel, *Mytilus galloprovincialis* is inhibited by copper ions (Viarengo *et al.*, 1981). In each of these studies, FAA transport is directly across the external epithelia, so there is reasonable assurance that heavy metal ions have a direct affect on epithelial FAA transport. In contrast, the rate of FAA transport by intact filter-feeding mollusks is highly dependent upon the rate of water transport (Wright and Stephens, 1978), so there is a possibility that reductions in uptake rate may be a secondary effect of a lowering of water pumping rate.

The data suggest that although there is a decrease in glycine uptake rate in the presence of zinc, the volume specific glycine transport remains constant. This suggests that the primary action of zinc may be the reduction of water transport by the oyster, and that the reduction of glycine uptake may be a secondary effect of the lowered water pumping rate. The presence of copper generally lowers the volume specific transport of glycine. This suggests that the inhibition of glycine transport at the epithelial carrier sites is greater than the inhibition of water transport. The data suggest that there may be some inhibition of water transport by copper, especially in high (>32 mg/l) concentrations. This observation is consistent with the observation of Brown and Newell (1972) that ciliary action on the gills of the mussel *Mytilus edulis* is inhibited in the presence of copper.

The presence of cadmium apparently increases the volume specific transport of glycine. This is a surprising result in that the concentration of glycine supplied in the media is 1 μM and the volume specific transport is 2.14 μmol/l in 128 mg/l Cd^{2+}. The implication is that two glycine μmoles are transported per liter of water transported when only 1 μmole of glycine is available. It is known that cadmium ions in solution can complex amino acids, particularly glycine (Wang and Gilpin, 1983). Uptake of cadmium–amino acid complexes by the oysters may provide an explanation for the unexpected results. Using Michaelis–Menten kinetics analysis of glycine uptake by human erythrocytes Nguyen and Chien (1988) showed that the K_t values generally decreased with increasing cadmium concentration, implying an increased carrier affinity for leucine. They offered as a possible explanation for this phenomenon the transport of cadmium–leucine complexes. The present data showing increase of volume specific glycine transport in the presence of cadmium is consistent with this suggestion. The transport of cadmium–glycine complexes might be manifested as a volume specific transport rate that implies greater than 100% removal of the substrate. There is, however, a possible second explanation for the observed increase in volume specific glycine transport. In this study, clearance of colloidal carbon is taken as an index of water pumping rate. If the particle retention efficiency of the oyster is 100% efficient, then carbon clearance rate would be equal to the water pumping rate. Since the volume specific glycine transport rate is quantitatively quite close to the supplied concentration of glycine in control oysters, this is circumstantial evidence that carbon clearance is a good approximation of water pumping rate and that glycine uptake efficiency is high. There is a possibility that cadmium may act to reduce the retention efficiency of carbon. A 50% reduction of carbon clearance efficiency would imply a doubling of the amount of water transport required to account for an equivalent amount of carbon to be cleared by the oyster. If carbon clearance efficiency is reduced, it would be manifested in this analysis as an apparent increase in the volume specific glycine transport. Further studies are necessary to investigate the effects of cadmium on particle filtration efficiency, and the direct transport of metal-amino acid complexes.

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