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In Vitro Examination of Interactions Between Copper and Zinc Uptake via the Gastrointestinal Tract of the Rainbow Trout (*Oncorhynchus mykiss*)

Adeola A. Ojo · Sunita Rao Nadella · Chris M. Wood

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Abstract An in vitro gut sac technique was used to investigate whether reciprocal inhibitory effects occurred between Cu and Zn uptake in the gastrointestinal tract of the rainbow trout and, if so, whether there was regional variation among the stomach, anterior intestine, mid intestine, and posterior intestine in the phenomena. Metal accumulation in surface mucus and in the mucosal epithelium and transport into the blood space were assayed using radiolabeled Cu or Zn at environmentally realistic concentrations of 50 μmol L^{-1} in the luminal saline, with 10-fold higher levels of the other metal (nonradioactive) as a potential inhibitor. Zn transport rates were generally higher than Cu transport rates in all compartments except the stomach, where they were lower. High [Zn] reduced Cu transport into the blood space in the mid and posterior intestines by 67% and 33%, respectively, whereas high [Cu] reciprocally reduced Zn transport into the blood space in these same sections by 54% and 78%. No inhibitions occurred in either the anterior intestine or the stomach. In these segments, elevated concentrations of the other metal stimulated Cu and Zn transport into the blood space and/or the mucosal epithelium by 50–100%, possibly by displacement from intracellular binding sites, thereby raising local concentrations at other transport sites. None of the treatments affected metal accumulation in surface mucus. The results indicate that one or more shared high-affinity pathways (possibly DMT1) occur in the mid and posterior intestine, which transport both Cu and Zn. These pathways appear to be absent from the stomach and anterior intestine, where other transport mechanisms may occur.

A. A. Ojo · S. R. Nadella · C. M. Wood (✉)
Department of Biology, McMaster University,
1280 Main Street West, Hamilton, ON, Canada L8S 4K1
e-mail: woodcm@mcmaster.ca

Present Address:
A. A. Ojo
P.O. Box 470, Ilesha, Osun State, Nigeria

Introduction

For essential metals such as Cu and Zn, the importance of the diet as the prime source has long been recognized by aquacultural nutritionists (e.g., Ogino and Yang 1978, 1979; Knox et al. 1984; Kjoss et al. 2006). In recent years, there has also been increasing realization that dietary metal uptake, rather than waterborne metal uptake, may be of primary importance in dictating toxicological responses of fish in some field situations (e.g., Dallinger and Kautzky 1985; Clearwater et al. 2002; Meyer et al. 2005). This has stimulated extensive research into the mechanisms of metal uptake via the fish gastrointestinal tract (Bury et al. 2003), particularly for Cu (Clearwater et al. 2000; Handy et al. 2000; Burke and Handy 2005; Nadella et al. 2006a, 2007; Glover and Wood 2008) and Zn (Shears and Fletcher 1983; Glover and Hogstrand 2002a, b, 2003; Glover et. al. 2003 2004). The picture which has emerged so far is complex, with more than one pathway apparently present for each metal, and some evidence of interactions between the two metals.

Specifically, in rainbow trout, Nadella et al. (2007) reported that a 10-fold excess of Zn would substantially inhibit Cu uptake through a high-affinity Cu pathway in in vitro gut sac preparations of both the mid and the posterior intestine. Glover and Hogstrand (2003) reported that an equimolar concentration of Cu would inhibit Zn uptake through a high-affinity Zn pathway in a perfused whole-intestine preparation of the trout in vivo. These observations led both groups of authors to propose that shared Cu-Zn

uptake pathways exist. However, recent evidence suggests that the stomach as well as the mid and posterior intestine, are the major sites of high-affinity Cu uptake in the rainbow trout in vivo (Nadella et al 2006b). For two other nutrient metals, Ca and Mg, the stomach and the anterior intestine appear to predominate (Bucking and Wood 2007). Cu uptake mechanisms in the stomach and anterior intestine were not examined by Nadella et al. (2007), while Glover and Hogstrand (2003), in their studies on Zn uptake, used a very different whole-intestine preparation which excluded the stomach and did not differentiate the three sections of the intestine. Recent findings that Cd uptake mechanisms differ between the stomach and the rest of the tract (Wood et al. 2006; Ojo and Wood 2008) suggest that regional differences may well occur.

With this background in mind, the present study asked two main questions. Can reciprocal inhibitory interactions of Zn and Cu be demonstrated in the same preparations, and do such effects occur in all four sections of the gastrointestinal tract of the rainbow trout (stomach, anterior, mid, and posterior intestine)? We employed a now well-characterized in vitro gut sac preparation which allows metal uptake to be measured in a relatively short period (typically 2–4 h) during which transport rates are stable and to be partitioned into mucus-bound, mucosal epithelium, and blood space components (Nadella et al. 2006b, 2007; Ojo and Wood 2007, 2008), thereby casting additional light on mechanisms involved. The resulting information on Cu and Zn transport mechanisms in the trout gut has environmental relevance in two contexts: (i) trace metal nutrition, in both wild fish and hatchery-reared salmonids, and (ii) toxicology of metals from the diet to fish, a pathway which is now believed to be even more important than the waterborne route in many field situations (Clearwater et al. 2002; Meyer et al. 2005).

Materials and Methods

Experimental Animals

Adult rainbow trout (*Oncorhynchus mykiss*; ~250 g, 30-cm total length; $N = 40$) were purchased from Humber Springs Fish Hatchery (Orangeville, ON, Canada). At McMaster University, they were held in 500-L tanks with flowing aerated and dechlorinated Hamilton city tap water (11–13°C) from Lake Ontario (approximate ionic composition, mmol.L^{-1}: 0.5 [Na^+], 0.7 [Cl^-], 1.0 [Ca], 0.2 [Mg^{2+}], and 0.05 [K^+], pH 7.8–8.0, dissolved organic carbon ~3 mg C L^{-1}, hardness ~140 mg.L^{-1} as $CaCO_3$). The fish were fed five times per week with a commercial trout chow (Martins Mills Inc. Elmira, ON, Canada) at a ratio of 1% body weight per feeding. Feed composition included 41% crude protein, 11% crude fat, 3.5% crude fiber, 1% calcium, 0.85% phosphorus, 0.45% sodium, 6800 IU kg^{-1} vitamin A, 100 IU kg^{-1} vitamin D2, and 80 IU kg^{-1} vitamin E. Measured metal concentrations were 27 μg g^{-1} copper, 173 μg g^{-1} zinc, 0.26 μg g^{-1} cadmium, 10 μg g^{-1} lead, 0.05 μg g^{-1} silver, and 3.9 μg g^{-1} nickel. Feeding was suspended 3 days prior to experiments.

In vitro Gut Sac Preparations

Methods identical to those described by Ojo and Wood (2007) were used to make gut sac preparations of the stomach, anterior intestine (including pyloric caecae), mid intestine, and posterior intestine. After being filled with the appropriate solutions, the sacs were weighed (0.1-mg accuracy), then transferred into 50-ml Falcon tubes (for the stomach and anterior intestine) or 15-ml Falcon tubes (for the mid and posterior intestine) for incubation in metal-free serosal saline. This external saline was bubbled constantly with a 99.5% O_2, 0.5% CO_2 gas mixture to maintain typical in vivo PCO_2 levels in venous blood of approximately 3.75 Torr. The same modified Cortland saline (Wolf 1963) as employed by Ojo and Wood (2007), with Cl^- replaced by SO_4^{2-} to avoid metal precipitation when Cu or Zn concentrations were increased 10-fold, was used for both inside (luminal) and outside (serosal) surfaces of the sacs in all experiments. At least for Cu, Nadella et al. (2007) have shown that this saline yields the same uptake rates in sac preparations as for traditional chloride-based saline. Composition (mmol L^{-1}) was 66.5 Na_2SO_4, 2.5 K_2SO_4, 1 $CaSO_4$, 1.9 $MgSO_4 \cdot 7H_2O$, 1.9 $NaHCO_3$, 2.9 $NaH_2PO_4 \cdot H_2O$, 5.5 glucose, pH 7.4. Osmolality was adjusted to 276 mOsm kg^{-1} by adding mannitol. In all experiments, this saline was used on the serosal surface, while the same saline spiked with the appropriate radiolabeled metal was used on the luminal surface.

The metal (Cu or Zn) for which the transport was being studied in a particular experiment was present at a total concentration of 50 μmol L^{-1} in the luminal saline, and was added in the form of $Cu(NO_3)_2$ or $ZnSO_4$, appropriately radiolabeled with a trace amount of either $^{64}Cu(NO_3)_2$ (McMaster Nuclear Reactor, Hamilton, ON, Canada) or $^{65}ZnCl_2$ (Los Alamos National Laboratory, Los Alamos, NM, USA) at a radioactivity of >1 μCi ml^{-1}. In inhibition trials, a 10-fold higher concentration (500 μmol L^{-1}) of the other metal was added as $Cu(NO_3)_2$ or $ZnSO_4$. The pH of the luminal saline was checked and set back to 7.4 after metal addition using NaOH. Samples of all salines were saved for verification of initial radioactivity and metal concentrations. The temperature was maintained at 11–13°C. Stomach gut sacs were incubated for 4 h, and intestinal gut sacs for 2 h. The longer time for stomach sacs was to improve accuracy, as transport rates were generally lower in this segment.

At final sampling, gut sacs were reweighed to ensure that fluid transport rates were within previously recorded ranges (Ojo and Wood 2007 2008) as a precaution to detect leaks. Samples of final serosal and luminal salines were taken for counting. The sacs were then cut open, washed in 5 ml of modified Cortland saline followed by 5 ml of 1 mmol L^{-1} EDTA disodium salt solution, and then blotted dry with a small piece of paper towel. A glass slide was used to gently scrape off the mucosal epithelia (i.e., the enterocytes), leaving behind the submucosa, muscle layers, and serosa, collectively referred to here as the "muscle layer." The exposed surface area of each segment was measured using graph paper, so as to allow expression of all transport rates per unit surface area.

Total radioactivities in three fractions were recorded. (i) The wash solutions plus blotting paper represented the "mucus-bound fraction." (ii) The mucosal epithelial scrapings represented metal that had been absorbed across the apical surface of the enterocytes but not exported to the blood. (iii) The muscle layer plus the serosal saline comprised the "blood compartment," representing metal that had been exported across the basolateral surface of the enterocytes. This provided a conservative estimate of the actual amount of metal which had been absorbed—i.e., transported through the enterocytes into the blood compartment. In vivo, the great majority of this metal would be taken away by the enteric blood flow, but the isolated gut sacs in vitro lack such vascular perfusion so the metal moves on beyond the blood vessels to the muscle layers and serosal fluid. Nevertheless, any metal which accumulates in either of these compartments must first have entered the blood space.

Analytical Techniques, Calculations, and Statistics

Concentrations of Cu and Zn in the luminal salines were measured by graphite furnace atomic absorption spectrophotometry (GFAAS; Varian Spectra AA-20 with graphite tube atomizer [GTA-110]; Mulgrave, Australia) and flame atomic absorption spectrophotometry (FAAS; Varian Spectra- 220 FS; Mulgrave), respectively. Calibration employed commercial Cu and Zn standards from Fisher Scientific (Toronto, ON, Canada). National Research Council of Canada (Ottawa, ON, Canada)-certified analytical standards run at the same time were within the specified range.

The gamma radioactivities of ^{64}Cu and ^{65}Zn in fluids and tissues were measured on a Minaxi-γ Auto gamma 5530 counter (Canberra Packard, Mississauga, ON, Canada) using energy windows of 433–2000 KeV for ^{64}Cu and 15–2000 KeV for ^{65}Zn The two radioisotopes were always used in separate experiments. ^{64}Cu was corrected for decay to a common reference time, because it has a very short half-life (12.9 h). Tests demonstrated that counting efficiencies were constant.

The rate of metal uptake into each of the three compartments ([i] mucus-bound, [ii] mucosal epithelium, and [iii] blood compartments) was calculated as

$$\text{Metal uptake rate} = \text{Compartment cpm}/(\text{SA} \cdot \text{ISA} \cdot \text{T})$$

where Compartment cpm represents the total ^{64}Cu or ^{65}Zn activity of the relevant compartment measured on the gamma counter, taking all volumes into account, SA is the mean measured specific activity of the luminal solution (cpm $nmol^{-1}$), ISA is the intestinal surface area ($cm^{2)}$, and T is time (h).

All data are reported as the mean ± 1 SE (N), where N represents the number of gut sac preparations (i.e., the number of animals). One-way analysis of variance (ANOVA) followed by a post hoc Bonferroni test was used to identify significant differences among the four segments (stomach, anterior intestine, mid intestine, posterior intestine) of the gastrointestinal tract in baseline control transport rates (at 50 µmol L^{-1} concentrations of Cu or Zn in the luminal saline). Within a segment, the same approach was used to identify significant differences in baseline control transport rates of Cu or Zn among the three compartments (blood space, mucosal epithelium, mucus binding). Differences between baseline control transport rates of Cu and Zn within a particular compartment and segment were made by Student's unpaired, two-tailed t-test. Similarly, as all competition experiments involved simple and independent comparisons of experimental versus separate control treatments, Student's unpaired, two-tailed t-test was employed. A significance level of $p < 0.05$ was used throughout.

Results

Baseline Cu and Zn Transport Rates

Cu and Zn at luminal concentrations of 50 µmol L^{-1} exhibited different patterns in their absolute transport rates and their partitioning among fractions in the four sections of the tract, with generally higher overall rates for Zn (Table 1).

Area-specific Cu transport into the blood space (the sum of serosal fluid + muscle components) was greatest in the anterior intestine, two- to fourfold higher than in the mid and posterior intestine, and sixfold higher than in the stomach (Table 1). Rates of Cu binding to mucus were approximately the same across all sections. These were similar in magnitude to blood compartment accumulation in the mid and posterior intestine, and therefore respectively about half the blood space value in the anterior intestine, and threefold higher than the blood space value in

Table 1 Baseline rates of Cu and Zn transport (at 50 μmol L^{-1} each, separately, in the luminal saline) in various compartments and segments of the gastrointestinal tract (mean ± 1 SE; $N = 9$–10)

	Blood space (nmol cm^{-2} h^{-1})	Mucosal epithelium (nmol cm^{-2} h^{-1})	Mucus binding (nmol cm^{-2} h^{-1})
Cu Stomach	$0.0121^{A,a}$	$0.0059^{A,a}$	$0.0443^{B,a}$
	±0.0021	±0.0008	±0.0061
Anterior intestine	$0.0795^{A,b}$	$0.0054^{B,a}$	$0.0396^{C,a}$
	±0.0110	±0.0007	±0.0078
Mid intestine	$0.0230^{A,B,a}$	$0.0173^{A,b}$	$0.0385^{B,a}$
	±0.0053	±0.0043	±0.0048
Posterior intestine	$0.0361^{A,a}$	$0.0033^{B,a}$	$0.0263^{A,a}$
	±0.0057	±0.0007	±0.0030
Zn			
Stomach	$0.0045^{A,a*}$	$0.0021^{A,a*}$	$0.0072^{A,a*}$
	±0.0005	±0.0004	±0.0073
Anterior intestine	$0.1415^{A,a,b}$	$0.0130^{A,b*}$	$0.0563^{A,a,b}$
	±0.0667	±0.0024	±0.0135
Mid intestine	$0.2132^{A,b*}$	$0.0362^{B,c*}$	$0.1049^{B,b*}$
	±0.0268	±0.0056	±0.0204
Posterior intestine	$0.1880^{A,b*}$	$0.0329^{B,c*}$	$0.0860^{B,b*}$
	±0.0304	±0.0139	±0.0195

Note: Within a row, means followed by the same superscript capital letter are not significantly different ($p > 0.05$) between compartments (blood space, mucosal epithelium, mucus binding). Within a column for a particular metal, means sharing the same superscript lowercase letter are not significantly different ($p > 0.05$) between segments (stomach, anterior intestine, mid intestine, posterior intestine). * Significant difference ($p < 0.05$) from respective Cu value

the stomach, where mucus binding represented the largest overall fraction. Cu accumulation in the mucosal epithelium was by far the smallest fraction in each section except the mid intestine, where it was comparable to transport into the blood compartment.

In contrast, rates of area-specific Zn transport into the blood space were generally higher than Cu transport in every section of the intestine but lower than Cu transport in the stomach (Table 1). These Zn transport rates into the blood space were approximately the same in the anterior, mid intestine, and posterior intestines, in contrast to the pattern of Cu transport, where the anterior intestine dominated. Rates of Zn accumulation in mucus were the next largest fractions. Accumulation of Zn in the mucus fraction in the stomach was again much lower than for Cu but did not differ greatly among the other sections, where rates were comparable to those for Cu. Zn accumulation in the mucosal epithelium was by far the smallest fraction in all sections (in this respect, similar to Cu). However, as for the blood compartment, absolute values of Zn accumulation in the mucosal epithelium were higher than for Cu in all three sections of the intestine but lower than for Cu in the stomach (Table 1).

The Influence of High [Zn] on Cu Absorption

A 10-fold higher concentration of Zn (500 μmol L^{-1}) exerted significant inhibitory effects against Cu transport into the blood space, by 67% at the mid intestine (Fig. 1c) and by 33% at the posterior intestine (Fig. 1d). There was also a 60% inhibition of Cu accumulation in the mucosal epithelium in the mid intestine (Fig. 1c) but not in the posterior intestine (Fig. 1d). Notably, there was no inhibitory effect of high [Zn] on Cu transport in the stomach (Fig. 1a) or anterior intestine (Fig. 1b). Indeed, in the latter, there was a significant 75% stimulation of Cu transport into the blood space, as well as 50% stimulation of Cu accumulation in the mucosal epithelium of the stomach (Fig. 1b). High [Zn] had no effect on Cu binding to mucus in any of the four segments (Fig. 1a–d).

The Influence of High [Cu] on Zn Absorption

The effects of high [Cu] on Zn transport largely mirrored the effects of high Zn on Cu transport. Thus 500 μmol L^{-1} Cu significantly inhibited Zn transport into the blood space by 54% at the mid intestine (Fig. 2c) and by 78% at the posterior intestine (Fig. 2d), but was without effect on accumulation in the mucosal epithelia. There was no inhibitory effect of high [Cu] on Zn transport in the stomach (Fig. 2a) or anterior intestine (Fig. 2b). Indeed, in the stomach, 500 μmol L^{-1} Cu caused a significant 55% stimulation of Zn transport into the blood space and an approximate doubling of Zn accumulation in the mucosal epithelium (Fig. 2a). High [Cu] had no effect on Zn binding to mucus in any of the four segments (Fig. 2a–d).

Discussion

The present results provide clear answers to the two objectives of the study, in showing that there are reciprocal inhibitory effects of Zn on Cu transport, and of Cu on Zn

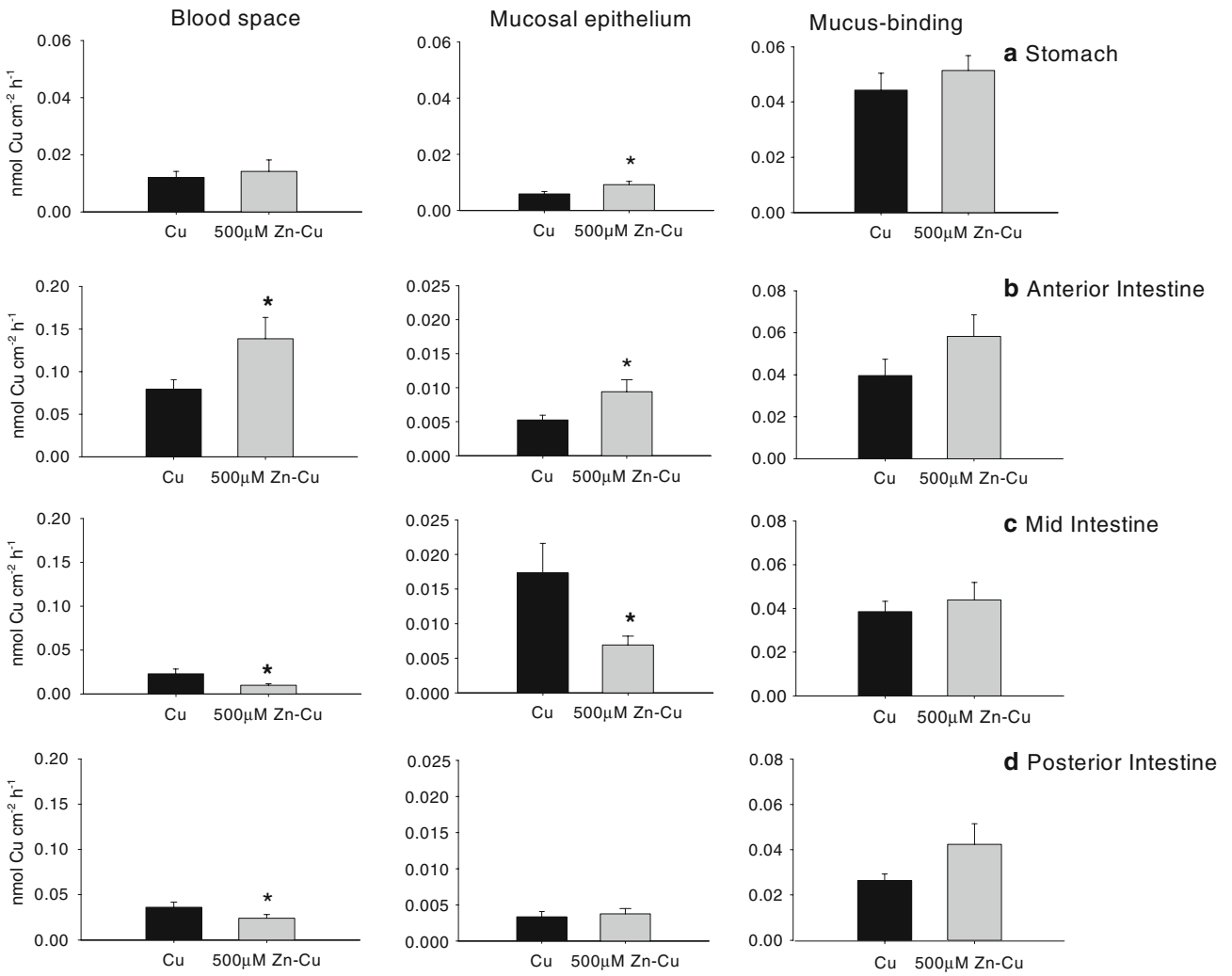

Fig. 1 The influence of luminal [Zn] (500 µmol L^{-1}) on the rates (nmol cm^{-2} h^{-1}) of Cu transport (at 50 µmol L^{-1} in the luminal saline) in (**a**) the stomach, (**b**) the anterior intestine, (**c**) the mid intestine, and (**d**) the posterior intestine. Appearance in blood space (i.e., serosal fluid plus muscle) is shown in the left-hand panels, appearance in mucosal epithelium in the middle panels, and appearance in the mucus-bound fraction in the right-hand panels. Mean (±SE); $N = 9$ or 10 for each treatment. *Significant difference within a panel ($p < 0.05$)

transport, in the same trout digestive tract preparations, and that these effects are confined to the mid and posterior intestines. As such, they tie together results from two very different preparations: the findings of Nadella et al. (2007) that Zn antagonizes Cu uptake in identical mid and posterior intestinal sacs in vitro and the findings of Glover and Hogstrand (2003) that Cu antagonizes Zn uptake in a perfused whole-intestine preparation of the trout in vivo.

In both cases, the antagonistic effects were seen against low, environmentally realistic concentrations (50 µmol L^{-1}) of Cu and Zn in the chyme and, therefore, likely involved the high-affinity transport system(s) for each metal. Nadella et al. (2006b) recorded dissolved Cu levels of 3–63 µmol L^{-1} in chyme from various sections of the gastrointestinal tract in trout fed on the same diet as used here. We are aware of no comparable measurements for Zn in trout chyme, but in a literature survey of other teleosts, Glover et al. (2004) tabulated values of 18 to 300 µmol L^{-1}. In trout mid and posterior intestine sacs in vitro, Nadella et al. (2006a) reported that K_M values (affinity constants) for Cu transport were in the range of 32–79 µmol L^{-1}, whereas K_M values of 57 µmol L^{-1} in brush border membrane vesicles of enterocytes in vitro (Glover et al. 2003) and 309 µmol L^{-1} in whole perfused trout intestine in vivo (Glover and Hogstrand 2002) have been documented for Zn transport. For both metals, there are also reports of much lower-affinity systems (i.e., with much higher K_M's: Cu [Burke and Handy 2005; Nadella et al. 2006a; Glover and Wood 2008]; Zn [Glover et al. 2003 2004]).

It would be naïve to suggest that all of the Cu and Zn uptake measured in the mid and posterior intestine, or even just the reciprocally sensitive portion, occurred through a

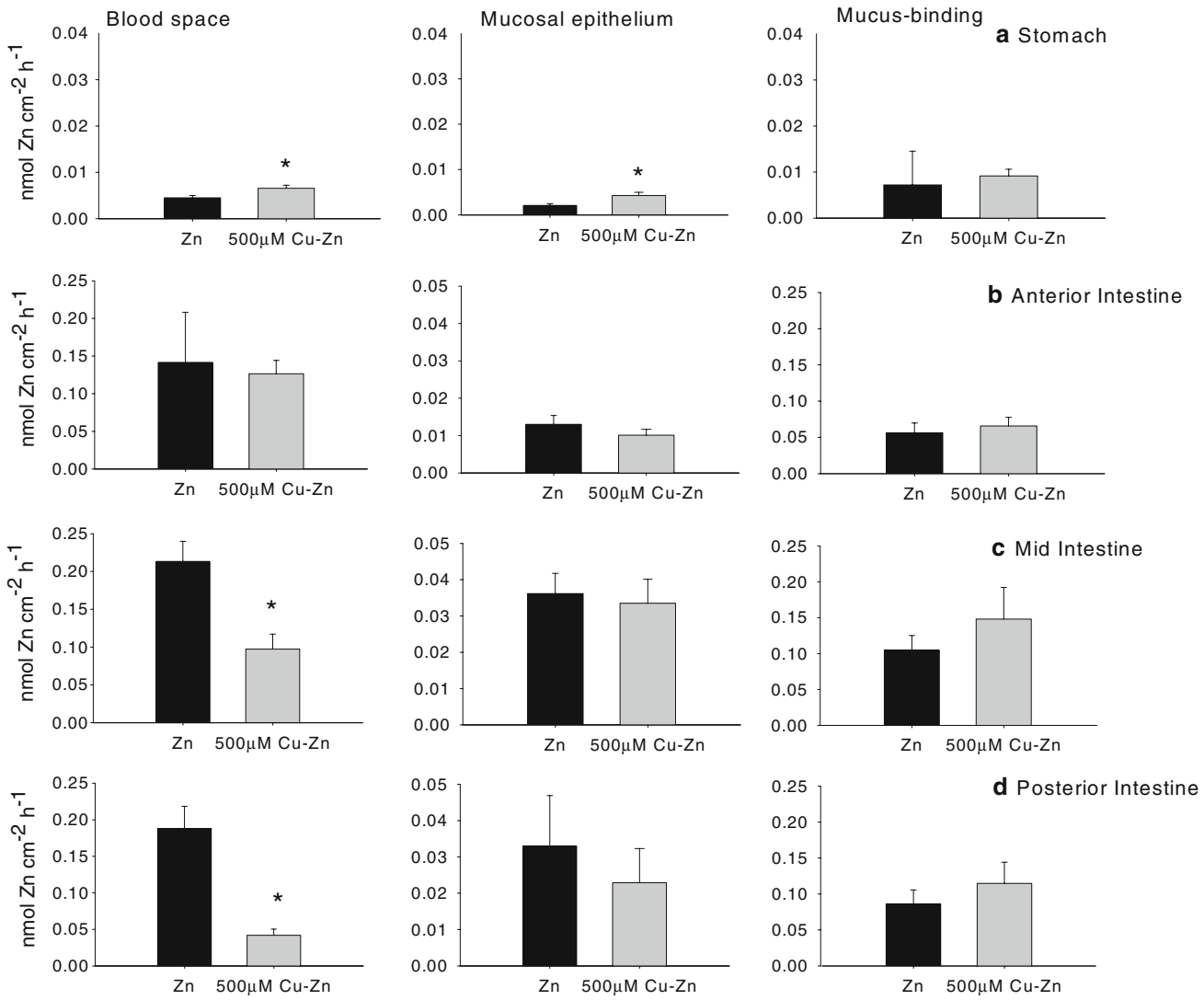

Fig. 2 The influence of luminal [Cu] (500 µmol L^{-1}) on the rates (nmol cm^{-2} h^{-1}) of Zn transport (at 50 µmol L^{-1} in the luminal saline) in (**a**) the stomach, (**b**) the anterior intestine, (**c**) the mid intestine, and (**d**) the posterior intestine. Appearance in blood space (i.e., serosal fluid plus muscle) is shown in the left-hand panels, appearance in mucosal epithelium in the middle panels, and appearance in the mucus-bound fraction in the right-hand panels. Mean (±SE); $N = 8$ for each treatment. Asterisk indicates significant difference within a panel ($p < 0.05$)

single pathway, in view of the many different transport systems described for Cu (e.g., DMT1, Ctr1, histidine coupled, Na^+ coupled) and Zn (e.g., DMT1, several Ca^{2+} channels, ZIP, histidine-coupled) in the gastrointestinal tract of mammals. It is quite possible that several different pathways were involved. Indeed, there is now molecular evidence (e.g., DMT1 [Dorschner and Phillips 1999; Donovan et al. 2002; Bury et al. 2003; Cooper et al. 2006, 2007; Ctr1 [Mackenzie et al. 2004]; Ca^{2+} channels [Qiu and Hogstrand 2004; Shahsavarini et al. 2006]; ZIP [Qiu et al. 2005]) and/or physiological evidence (e.g., DMT1 [Cooper et al. 2006, 2007; Nadella et al. 2007]; Ctr1 [Burke and Handy 2005]; Ca^{2+} channels [Larsson et al. 1998; Wood et al. 2006]; Na^+-coupled Cu transport [Nadella et al. 2007]; histidine-coupled Cu transport [Nadella et al. 2006a; Glover and Wood 2008]; histidine-coupled Zn transport [Glover and Hogstrand 2002b; Glover et al. 2003]) for the occurrence of many of them in fish.

However, in view of the lack of added histidine in our preparations, the reported high specificity of Ctr1 as only a Cu transporter (Lee et al. 2002), and the lack of inhibition of Zn transport by high [Ca] in identical trout gut sac preparations (Ojo and Wood 2008), our attention is particularly drawn to DMT1. DMT1 is a "promiscuous" divalent metal transporter whose primary role is probably Fe transport, but which is also known to transport Cu and Zn (Gunshin et al. 1997; Bury et al. 2003). Nadella et al. (2007) reported that 500 µmol L^{-1} Fe (tested against 50

μmol L^{-1} Cu) inhibited Cu transport into the blood compartment by 80% in the mid intestine and 20% in the posterior intestine in identical trout gut sac preparations, somewhat parallel to 75% and 40% inhibitions, respectively, by 500 μmol L^{-1} Zn. In the present study, 500 μmol L^{-1} Zn similarly inhibited Cu transport by 67% and 33%, respectively. Collectively, these results suggest that DMT1 (or other Zn- and Fe-sensitive mechanisms) is more important in Cu transport in the mid intestine than the posterior intestine. Interestingly, the reverse pattern (54% inhibition in mid intestine, 78% inhibition in posterior intestine) was seen when 500 μmol L^{-1} Cu was tested against Zn transport (50 μmol L^{-1} Zn), suggesting different contributions of this mechanism(s) to Zn transport in the two segments. Recently, using a *Xenopus* oocyte expression system, Cooper et al. (2007) showed that Fe transport by two isoforms of DMT1 cloned from rainbow trout gill could be inhibited by high [Zn], though high [Cu] was not tested.

The present results indicate that the shared Cu-Zn mechanism(s) does not occur in either the stomach or the anterior intestine. There is an analogy to Cd here, which presents a very different transport mechanism in the stomach relative to the rest of the tract (Wood et al. 2006; Ojo and Wood 2008). In the rainbow trout in vivo, the stomach is an important site of net Cu absorption, whereas the anterior intestine is a site of net Cu secretion, but this is likely because pyloric cecal and biliary secretions (Cu efflux) outweigh Cu influx in this region (Nadella et al. 2006a). Similar patterns have been seen for two other divalent metals (Ca, Mg [Bucking and Wood 2007]). We are aware of no evidence as to the relative importance of different segments in Zn uptake in trout in vivo. In the present in vitro study, Zn transport rates into the blood space were approximately the same in the anterior, mid intestine, and posterior intestines, but very low in the stomach. In the winter flounder, using in vivo gut sacs, Shears and Fletcher (1983) reported the highest rates of Zn absorption from a standard incubation medium in the anterior intestine and the lowest in the stomach. However, uptake from a constant concentration does not inform about relative uptake from natural meals in vivo, because for Cu (Nadella et al. 2006a), Ca, Mg (Bucking and Wood 2007), and Cd (Baldisserotto et al. 2005), concentrations available for uptake are manyfold higher in the acidic stomach chyme than in the basic intestinal chyme. This appears to tip the balance in favor of high uptake rates at the stomach.

Rather than reciprocal inhibition, there was some evidence of reciprocal stimulation of transport by Cu and Zn in the stomach and anterior intestine. Specifically, high [Zn] stimulated Cu accumulation in the mucosal epithelium of the stomach and Cu transport into the blood compartment in the anterior intestine. Conversely, high [Cu] stimulated Zn transport into both the mucosal epithelium and the blood compartment of the stomach. While unexpected, such effects are not entirely unprecedented. For example, Ojo and Wood (2008) reported that high [Ca] also stimulated Zn transport in the stomach. Indeed, Glover and Hogstrand (2003) reported that equimolar [Cu] stimulated Zn uptake into the mucosal epithelium of an in vivo perfused intestinal preparation of the trout. Glover and Hogstrand (2003) and Glover et al. (2004) did not study the stomach, but reported a stimulation of Zn uptake by elevated [Ca] in both the perfused intestinal preparation and the isolated intestinal apical membranes (brush border membrane vesicles) of trout. Glover et al. (2004) suggested that such stimulatory effects could be due to the high level of exogenous metal displacing the substrate metal from binding sites, thereby increasing its local concentration at specific uptake sites. Given the lack of changes in the mucus-bound fractions in the present study, such sites would likely be within the mucosal epithelium. As suggested by Glover and Hogstrand (2003), one possibility for such sites could be metallothionein molecules, which bind both Cu and Zn.

In summary, there are clear reciprocal inhibitory effects between high-affinity Cu and Zn transport in the mid and posterior intestines of rainbow trout, which add to the growing body of knowledge on metal transport mechanisms in these sections in fish. These effects are not seen in the stomach and anterior intestine, sections which may be quantitatively more important in Cu and Zn absorption in vivo. Indeed, virtually nothing is known regarding the mechanisms of Cu and Zn transport in these two segments, presenting an important area for future research.

The information collected here, together with other recent studies on nutrient and nonnutrient metal uptake via the gastrointestinal tract in freshwater fish (see Introduction), contributes to the growing body of knowledge about mechanisms of metal transport and interaction in the teleost gut. In addition to its obvious importance for nutritional physiology in aquacultured and wild fish, such information is vital for the development of predictive models for dietary metal toxicity. A comparable effort to develop a physiological understanding of metal interactions and transport at the fish gill over several decades was integral to the development of the Biotic Ligand Model (BLM) to predict waterborne metal toxicity on a site-specific basis (for historical reviews see Paquin et al. [2002]; Niyogi and Wood [2004]). This approach has fundamentally changed the regulatory approach to waterborne metals (see Reiley 2007); it is hoped that studies such as the present will eventually lead to a parallel gut BLM to predict dietary metal toxicity.

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