Effect of hydrogen ions and inorganic complexing on the uptake of copper by the brine shrimp *Artemia franciscana*

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ABSTRACT: The effect of hydrogen ions and inorganic complexing on copper uptake in the brine shrimp *Artemia franciscana* has been studied in chemically defined saltwater solutions. Uptake increases with decreasing hydrogen ion concentration and decreases with increasing carbonate complexation. A simple non-linear model that combines the effect of hydrogen ions on the transport of the metal across the solution-body interface and the effect of hydrogen ions and complexation on the speciation of the metal provided a close functional description of the observed variation in copper uptake by brine shrimp.

INTRODUCTION

The uptake and toxicity of copper in aquatic organisms is largely determined by the physical and chemical speciation of the metal in the environment and is related to the concentration of the free cupric ion in the solution (Borgmann 1983, Campbell & Stokes 1985, Wangersky 1986, Luoma 1989). In a saltwater environment most of the copper is complexed by organic and inorganic ligands. Only a small fraction of the total amount of copper exists as the free cupric ion (Waite & Morel 1983, Van den Berg 1984, Moffett & Zika 1987, Sunda & Hanson 1987, Turner & Whitfield 1987, Coale & Bruland 1988).

Experimental work carried out in saltwater solutions, where changes in the hydrogen ion concentration are minimal, have shown that complexation of copper with organic ligands decreases the uptake and toxicity of copper (Sunda & Guillard 1976, Knezovich et al. 1981, Zamuda & Sunda 1982, Ahsanullah & Florence 1984, Zamuda et al. 1985, Blust et al. 1986, Florence & Stauber 1986, Sunda et al. 1987). This effect is usually explained as resulting from decreases in the concentration of the cupric ion. Studies carried out in freshwater solutions, where changes in the hydrogen ion concentration are more pronounced, have also shown that complexation of copper with organic ligands decreases the uptake and toxicity of copper (Dodge &

Theis 1979, Guy & Kean 1980, Borgmann & Ralph 1983, Buckley 1983, Starodub et al. 1987). However, it has also been shown that the uptake and toxicity of copper increases with decreasing hydrogen ion concentration and decreasing cupric ion concentration (Andrew et al. 1977, Howarth & Sprague 1978, Chakoumakos et al. 1979, Miller & Mackay 1980, Cusimano et al. 1986, Laurén & McDonald 1986, Starodub et al. 1987). Under these circumstances the direct relation between the concentration of the cupric ion in solution and the uptake and toxicity of the metal in the aquatic organism is lost.

The first step in the uptake of copper from solution by an aquatic organism probably involves interaction with a membrane-embedded transport system to form a temporary association which carries the metal across the interface. Most such systems are sensitive to the binding of other positively charged species, most notably the hydrogen ion. Although the pH of saltwater remains relatively constant within space and time, the pH at the solution-body interface can vary considerably due to the activity of the specialised tissues which line the interface and create an intermediate environment differing from the external environment. Hydrogen ions may alter the availability of copper in 3 distinct ways: (1) changing the speciation of the metal in the solution; (2) modulating the activity of the metal transport system; and (3) affecting physiological processes

which either directly or indirectly influence the uptake process (e.g. altering membrane potentials, ionic and osmotic regulation, energy metabolism) (Williams 1981, Simkiss & Taylor 1989, Viarengo 1989).

To understand how these chemical and biological processes influence the availability of copper to organisms it is necessary to determine the combined effect of changes in the hydrogen ion concentration and in the inorganic speciation of copper on the uptake of the metal by multicellular organisms that live in saltwater environments. For this purpose we studied the combined effect of hydrogen ions and inorganic complexing on copper uptake in the brine shrimp *Artemia franciscana*.

MATERIALS AND METHODS

Brine shrimp. Dried *Artemia franciscana* cysts from Great Salt Lake, Utah, USA were purchased from San Francisco Bay Brand, Newark, CA, USA. Cysts were hatched in a funnel-shaped plastic container filled with synthetic seawater (Wiegandt, Krefeld, Germany), and aerated from the bottom. The hatching suspension was illuminated by a fluorescent light tube. Hatching cyst density was 5 g l^{-1}. *Artemia* nauplii were harvested after 36 h. The larvae were grown from nauplii to adult in 150 l plastic rectangular air-water lift operated raceways filled with synthetic seawater. Shrimp were fed with a suspension of Yellow-Mix (Artemia Systems, Ghent, Belgium). They reached maturity after 3 to 4 wk and were used before they were 8 wk old. The methods for intensive culturing of brine shrimp have been described by Sorgeloos et al. (1983).

Experimental procedures. In this study of the biological availability of copper to brine shrimp we used a static test design. The accumulation of copper during 3 h of exposure was used as a measure of the biological availability of the metal. Experiments were conducted in a thermostated room at 25.0 ± 0.5 °C. One day before an experiment was run adult brine shrimp were collected from a batch culture and placed into clean medium to clear their guts. The composition of the chemically defined saltwater solution was (per liter) 23.50 g NaCl, 4.00 g Na_2SO_4, 0.680 g KCl, 0.196 g $NaHCO_3$, 1.470 g $CaCl_2 \cdot 2H_2O$, 10.78 g $MgCl_2 \cdot 6H_2O$ and 0.026 g H_3BO_3. The medium was prepared by dissolving the 7 analytical grade products (Merck p.a.) in deionised water. A dispersion of 0.1 mmol l^{-1} MnO_2 was added to the seawater to remove metals present in the analytical grade reagents. After an equilibration period of 24 h, the dispersion was filtered through a 0.2 μm membrane filter to remove the MnO_2 from the solution (Van den Berg & Kramer 1979). The pH of the solutions was controlled by incorporation of the zwitterionic buffers 2-(N-morpholino)ethanesulphonic acid

(MES) and N-2-hydroxyethylpiperazine-N'-2-ethanesulphonic acid (HEPES) to control the pH between 5.3 and 6.8 and between 6.8 and 8.3, respectively. These buffers form only weak complexes with divalent metal ions, and complexation of copper is negligible at the concentration of 10 mmol l^{-1} MES or HEPES used (Good et al. 1966). The pH of the test solutions was adjusted with HCl or NaOH as required and the media were aerated to promote equilibration of gases with the atmosphere. At equilibrium the total dissolved CO_2 concentration is determined by the pH of the solution and the partial pressure of CO_2 in the atmosphere. To control the total dissolved CO_2 concentration at fixed pH the solutions were aerated with synthetic air of known CO_2 concentration (i.e. 0 to 5.0 %). Dissolved O_2 concentration, total dissolved CO_2 concentration, pH and redox potential (pE) were measured to ensure that equilibrium conditions had established. Dissolved oxygen was measured with a polarographic oxygen electrode system (WTW OX191/EO90). Total dissolved CO_2 was measured with a gas-sensing CO_2 electrode (Ingold 152323000), after acidification of the water sample (pH < 4.8) in a sealed measuring vessel. The hydrogen ion activity was measured with a glass electrode (Ingold 104573002), and pH values expressed on a free hydrogen ion scale (Millero 1986). Redox potentials were measured with a wire type platinum electrode (Ingold 105003077).

Copper was added to the test solutions from a 0.1 mmol l^{-1} cupric ion stock [i.e. $Cu(NO_3)_2$]. In a first series of experiments the total concentration of copper in the test solutions ranged between 1 and 100 μmol l^{-1}. Higher concentrations of copper could not be used due to the limited solubility of the metal. In a second series of experiments the total concentration of copper in the test solutions was 5 μmol l^{-1}. Experiments were carried out in 0.5 l plastic beakers. The test solutions were aerated during the experiments to maintain equilibrium. Just before an experiment started about 50 brine shrimp were collected on a 250 μm screen, rinsed with clean medium and transferred to a beaker. After 180 min the beaker was removed and a few ml of the test solution sampled in a plastic vial and stored frozen at −20 °C until analysed for copper. The beaker was subsequently emptied over a 250 μm screen. The collected shrimp were rinsed with deionised water and divided into 5 plastic vials, dried for 24 h at 60 °C and stored in a dessication box until analysed for copper. All experiments were run at least in duplicate using brine shrimp from different batch cultures. For each treatment group 5 replicate samples were obtained. Dissolved O_2 concentration, total dissolved CO_2 concentration, pH, pE and total dissolved copper concentration were measured at the beginning and end of an experiment. Generally, all measured values remained within 10 % of initial values.

Chemical modelling. The equilibrium concentrations of the chemical species considered were calculated using the computer program SOLUTION (Blust et al. unpubl.), an adaptation of the program COMPLEX (Ginzburg 1976). This speciation model allows calculation of the composition of solutions in equilibrium with gas and solid phases. The model uses the ion-association concept, which invokes the existence of molecular species like free ions, ion-pairs and complexes. A thermodynamic stability constant data base was built, based on the model of Dickson & Whitfield (1981) for the major components and the models of Turner & Whitfield (1987) and Sharma & Millero (1988) for copper. For each ion-pair or complex species considered the stability constants listed for different ionic strengths were fitted to an interpolation function that has the form of an extended Debye-Hückel equation (Turner et al. 1981). The pE of the solutions was calculated from the empirical relation $pE = 17.6 - pH$ (Baas-Becking et al. 1960). The thermodynamic stability constants and concentration products at the ionic strength of the saltwater solution for all copper species included in the model are given in Table 1. Case specific input includes the total concentrations of the metals and ligands in the solution, the free hydrogen ion concentration, redox potential, temperature and the gas and solid phases that are maintained in equilibrium with the solution.

Metal analysis. Copper was measured by Graphite Furnace Atomic Absorption Spectrophotometry using a Perkin-Elmer 703 Spectrophotometer fitted with a Heated Graphite Atomiser HGA-500 and a deuterium arc background corrector. The method of stabilised temperature platform atomisation was used (Slavin et al. 1983). Saltwater solutions were diluted 5 times with water. To this solution a one-tenth volume of concentrated nitric acid was added. The solutions were analysed directly against matrix matched calibration standards. Biological material was dissolved with concentrated nitric acid in a microwave oven. The resulting digest was diluted with water and analysed against 10 % nitric acid calibration standards (Blust et al. 1988).

Statistical analysis. All sets of data were tested for homoscedasticity by the log-ANOVA test for homogeneity of variances and for normality by the Kolmogorov-Smirnov test for goodness of fit. Analysis of variance, single and multiple linear regression and non-linear regression methods were used for analysing the data. The T-method was used to make multiple comparisons among pairs of means. Significance levels of tests are indicated as follows: $\cdot$ $0.05 \geq p > 0.01$, $\cdot\cdot$ $0.01 \geq p > 0.001$, $\cdot\cdot\cdot$ $p \leq 0.001$. Statistical methods are as outlined in Snedecor & Cochran (1980) and Glantz & Slinker (1990).

RESULTS

Chemical speciation of copper

The complexation of copper in a chemically defined saltwater solution that does not contain organic ligands is controlled by the concentration of hydroxide and carbonate. The concentration of hydroxide is determined by the pH of the solution. The concentration of carbonate is determined by the partial pressure of CO_2 in the atmosphere and the pH of the solution. Changing the partial pressure of CO_2 in the atmosphere makes it possible to alter the carbonate concentration without changing the pH. Measurement of the pH and total dissolved CO_2 concentrations in the solutions suffices for calculation of the carbonate speciation in a chemically defined solution. Results of measurements concerning the effects of pH and CO_2 on the total dissolved CO_2 concentration in the solutions and the effects on the chemical speciation of copper are summarised in Figs. 1 to 4. In a basic environment copper is complexed with hydroxide and carbonate but towards acidic conditions the cupric ion and some covarying inorganic species (i.e. $CuCl^+$ and $CuSO_4^0$), become increasingly more important. For the same total dissolved CO_2 concentration, carbonate complexing is important in a basic, but not in an acidic environment.

Uptake of copper by brine shrimp

Results summarised in Fig. 5 show that the uptake of copper in brine shrimp increases linearly with the total concentration of the metal in the solution and

Table 1. Thermodynamic stability constants and concentration products for copper species. K: thermodynamic stability constant; Q: concentration product at I = 0.614

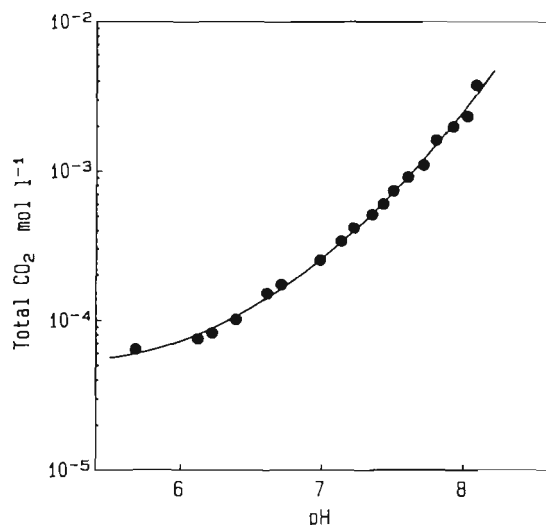| Species	Log K	Log Q
Cu^{2+}	–	–
$CuCl^+$	0.40	−0.02
$CuSO_4^0$	2.36	1.32
$CuOH^+$	6.01	5.61
$Cu(OH)_2^0$	11.75	10.83
$CuCO_3^0$	6.74	5.73
$CuHCO_3^+$	12.25	10.70
$Cu(CO_3)_2^{2-}$	10.57	9.27
Cu^+	2.61	2.12
$CuCl^0$	5.72	4.85
$CuCl_2^-$	8.03	7.21
$CuCl_3^{2-}$	7.36	6.96

Fig. 1. Measured total dissolved carbon dioxide concentration as a function of pH in a saltwater solution in equilibrium with air (sal. = 3.5 %, $pO_2 = 10^{-0.69}$ atm, $pCO_2 = 10^{-3.48}$ atm)
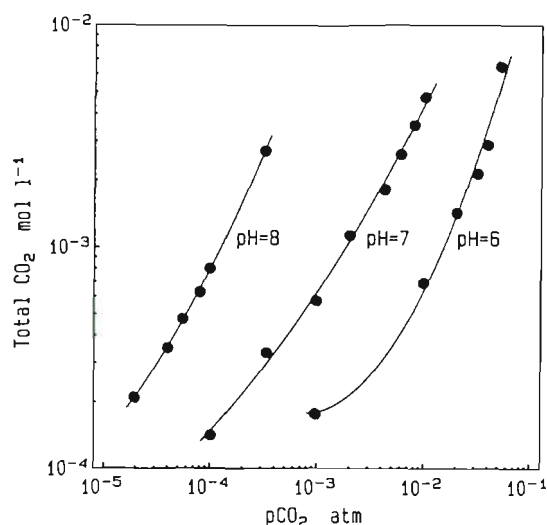

Fig. 3. Measured total dissolved carbon dioxide concentration as a function of pH and pCO_2 in a saltwater solution (sal. = 3.5 %, $pO_2 = 10^{-0.69}$ atm)
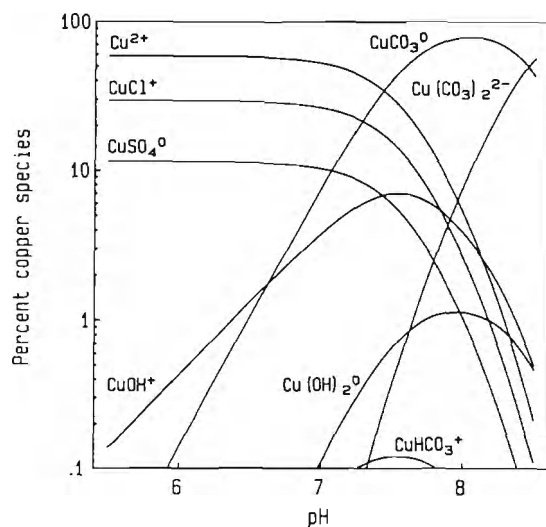

Fig. 2. Speciation model of copper as a function of pH in a saltwater solution in equilibrium with air (sal. = 3.5 %, $pO_2 = 10^{-0.69}$ atm, $pCO_2 = 10^{-3.48}$ atm)
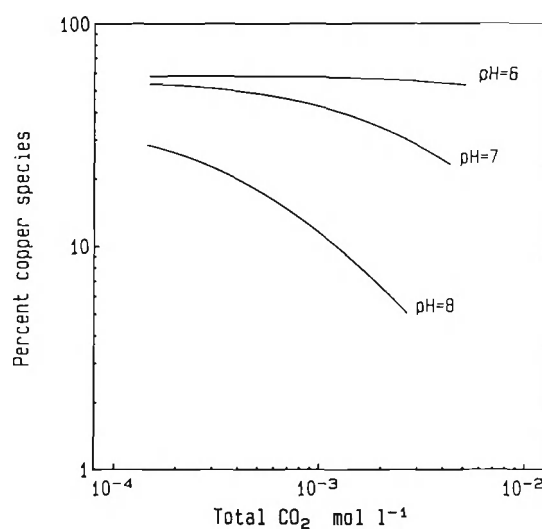

Fig. 4. Speciation model of copper as a function of pH and pCO_2 in a saltwater solution (sal. = 3.5 %, $pO_2 = 10^{-0.69}$ atm). For clarity only the concentration of the cupric ion is shown

that it is much higher in basic and neutral than in acid environments. Apparently, copper uptake does not saturate over the copper concentration ranges used (pH = 6, Cu_T = 10 to 100 µmol l^{-1}; pH = 7 and 8, Cu_T = 1 to 10 µmol l^{-1}). Over the total copper and pH range tested, copper uptake is positively correlated with the concentrations of Cu^{2+} (r = 0.66•••, n = 31), $CuOH^+$ (r = 0.93•••), and $CuHCO_3^+$ (r = 0.76•••).

To determine the effect of complexation of copper with hydroxide and carbonate on the availability of copper at different pH values, subsequent experiments were conducted at one total copper concentration of 5 µmol l^{-1}, 3 different pH values (6.0, 7.0 and 8.0), and different carbonate concentrations. The results summarised in Fig. 6 show that copper uptake increases with pH. Over the pH range tested, copper uptake in brine shrimp is positively correlated with the concentrations of $CuOH^+$ (r = 0.79•, n = 8), $Cu(OH)_2^0$ (r = 0.88••), $CuCO_3^0$ (r = 0.88••) and $CuHCO_3^+$ (r = 0.82•). Results summarised in Fig 7 show that copper uptake decreases with the complexation of copper with carbonate. This effect is most pronounced at
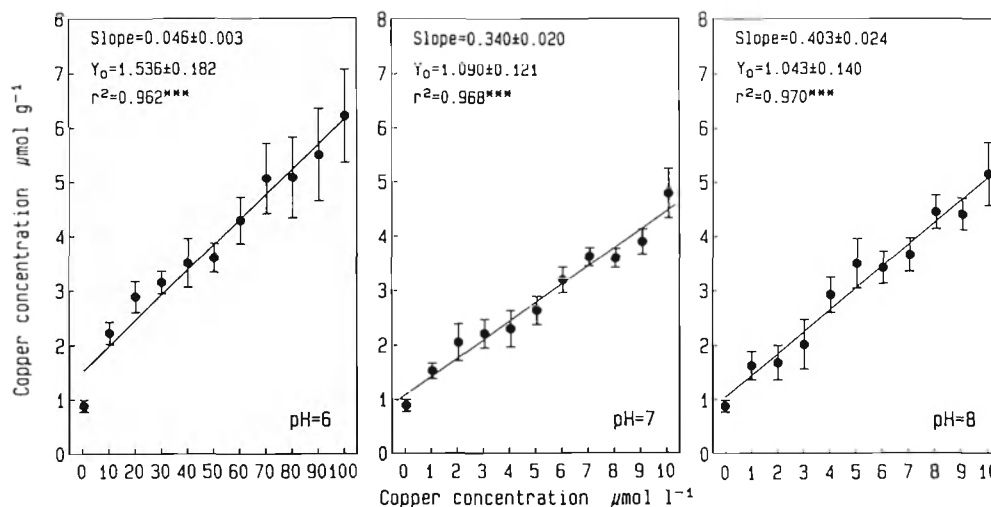
Fig. 5. *Artemia franciscana*. Uptake of copper at pH = 6.0, 7.0 and 8.0 as a function of total copper concentration over a 3 h period (sal. = 3.5 %, $pO_2 = 10^{-0.69}$ atm, $pCO_2 = 10^{-3.48}$ atm)
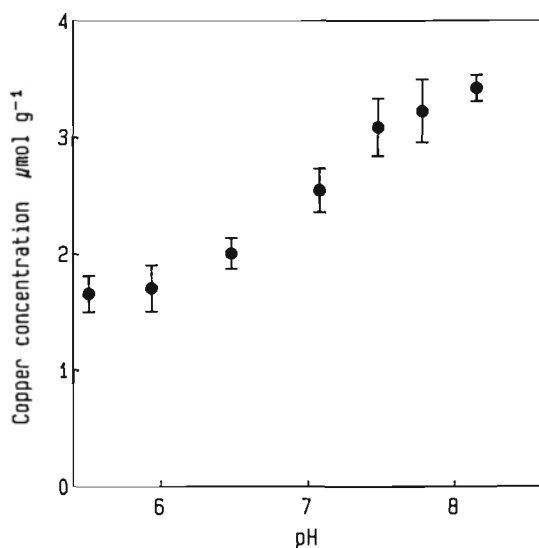

Fig. 6. *Artemia franciscana*. Uptake of copper as a function of pH over a 3 h period, exposed to a copper concentration of $5 \cdot 10^{-6}$ mol l^{-1} (sal. = 3.5 %, $pO_2 = 10^{-0.69}$ atm). Means are significantly different, p < 0.01

pH 8.0 and 7.0. At pH 6.0 complexation of copper with carbonate is negligible, though a moderate decrease in copper uptake with increasing total carbon dioxide was observed. Over the pH range tested, copper uptake is positively correlated with the concentrations of $CuOH^+$ (r = 0.98***, n = 21) and $Cu(OH)_2^0$ (r = 0.98***).

Analysis of the pooled results shows that overall, only the concentrations of the free cupric ion and the 2 cupric hydroxide species are positively correlated with the uptake of copper in brine shrimp. Results of the correlation analysis are summarised in Table 2.

Linear and non-linear modelling of copper uptake

Multiple linear regression analysis of the pooled results was used to explain the variation in copper uptake as the result of changes in the speciation of the metal on the uptake of a limited number of metal species. The linear equation for metal uptake assumes the form of a sum of terms. Each term is the product of a regression coefficient (RC) and the concentration of a metal species (MS). Taking into account the amount of copper already present in clean organisms (M_0), the equation for the total amount of copper in the organism (M_T) becomes $M_T = M_0 + RC_1 \cdot MS_1 + RC_2 \cdot MS_2 + \ldots$ Using this equation the analysis of the pooled results shows that most of the variation in copper uptake is explained when the free cupric ion and the 2 cupric hydroxide species are considered to be the metal species that are taken up. Results of the multiple linear regression analysis are summarised in Table 3a and Fig. 8a. It has to be noted that the 2 copper hydroxide species considered show a high degree of collinearity which implies that their separate contribution cannot be assessed in an unambiguous way.

Multiple non-linear regression analysis of the pooled results was used to explain the variation in copper uptake as the combined effect of changes in the speciation of the metal and of changes in the hydrogen ion concentration on the uptake of a limited number of metal species. The effect of the hydrogen ions on metal uptake is expressed as an effect on the ionisation of the metal transport system. The non-linear equation for metal uptake assumes the form of an equilibrium relation between the concentrations of the hydrogen ion, the copper species taken up and the ionised carrier. The fraction of ionised carrier is determined by the acid
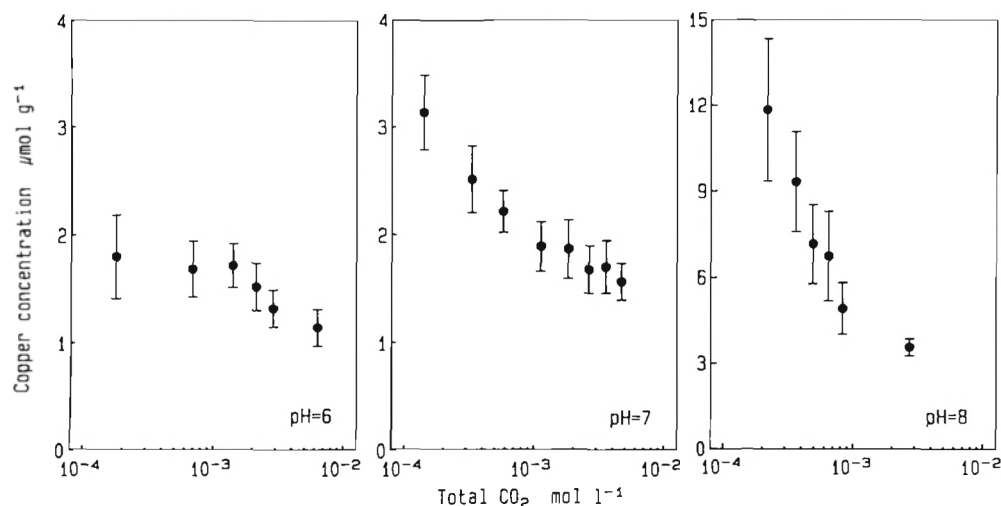
Fig. 7. *Artemia franciscana*. Uptake of copper at pH = 6.0, 7.0 and 8.0 as a function of total dissolved carbon dioxide concentration over a 3 h period, exposed to a copper concentration of 5×10^{-6} mol l^{-1} (sal. = 3.5 %, $pO_2 = 10^{-0.69}$ atm). Means are significantly different, $p < 0.01$

Table 2. Copper speciation and uptake in brine shrimp: pooled data correlation matrix (n = 58). [ns] Not significant; * $0.05 \geq p > 0.01$; ** $0.01 \geq p > 0.001$; *** $p \leq 0.001$

	Cu^{2+}[a]	$CuOH^+$	$Cu(OH)_2^0$	$CuCO_3^0$	$CuHCO_3^+$	$Cu(CO_3)_2^{2-}$
Uptake	0.283*	0.958***	0.810***	0.257[ns]	−0.061[ns]	0.131[ns]
Cu^{2+}	–	0.171[ns]	−0.242[ns]	−0.309*	0.549***	−0.241[ns]
$CuOH^+$	–	–	0.823***	0.285*	−0.094[ns]	0.080[ns]
$Cu(OH)_2^0$	–	–	–	0.520***	−0.279*	0.328*
$CuCO_3^0$	–	–	–	–	−0.102[ns]	0.886***
$CuHCO_3^+$	–	–	–	–	–	−0.182[ns]

[a] Cu^{2+}, $CuCl^+$, $CuSO_4^0$ vary together

dissociation constant (pK) of the ligand and the pH of the solution [i.e. $1/(1 + 10^{(pK - pH)})$]. Metal uptake is then proportional to the ratio of the fraction of available copper and the fraction of ionised carrier. To relate this ratio to metal uptake, it is necessary to introduce a coefficient (CF) which relates the sum of the concentrations of the metal species (SMS) in the solution to the concentration of copper taken up in the shrimps. Taking into account the amount of copper already present in clean organisms (M_0), the equation for the total amount of copper in the organism (M_T) becomes $M_T = M_0 + CF$ SMS $1/(1 + 10^{(pK - pH)})$. Using this equation analysis of the pooled results shows that most of the variation in copper uptake is explained when the acid dissociation constant of the transport system has a value of 8 and the cupric ion and the 2 cupric hydroxide species are considered to be the metal species that are taken up. Results of the multiple non-linear regression analysis are summarised in Table 3b and Fig. 8b.

Table 3. Copper uptake in brine shrimp. (a) Pooled data linear regression (R = 0.979***, n = 58); (b) pooled data non-linear regression (R = 0.961***, n = 58). B: partial regression coefficient; SE: standard error of partial regression coefficient; L_1, L_2: 95 % confidence limits for partial regression coefficient; Intercept: copper concentration in clean shrimp (M_0). Concentrations are in µmol l^{-1} for copper in solution and µmol g^{-1} for copper in shrimp

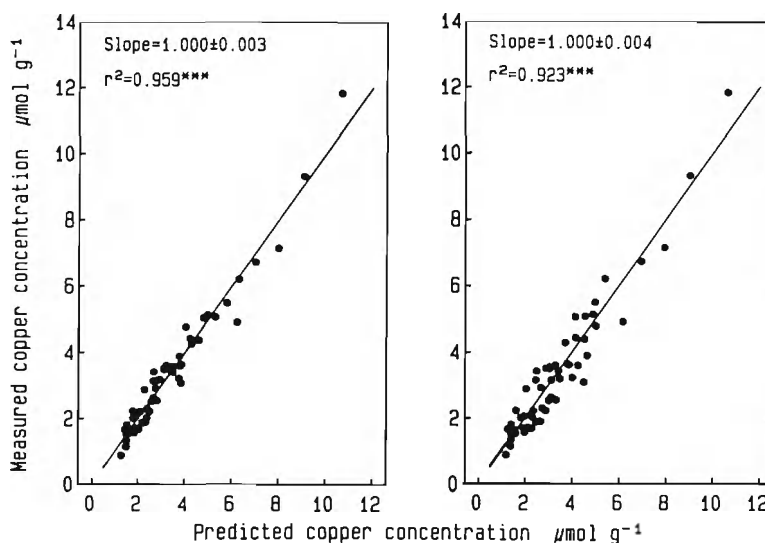| Variable	B	SE	L_1	L_2
(a) Linear				
Intercept	1.276***	0.091	1.094	1.458
Cu^{2+}	0.041***	0.006	0.030	0.053
$CuOH^+$	6.032***	0.678	4.673	7.391
$Cu(OH)_2^0$	12.895***	2.152	8.580	17.210
(b) Non-linear				
Intercept	1.175***	0.123	0.930	1.421
Coefficient	7.856***	0.712	6.429	9.283
pK	8.036***	0.063	7.911	8.161

Fig. 8. *Artemia franciscana*. Predicted versus measured concentrations of copper for (a) the linear and (b) the non-linear model

DISCUSSION

The results concerning the effect of different total copper concentrations on copper availability show that metal uptake is linear over the pH and concentration range used. This means that copper uptake is either (1) a simple diffusion process with the net rate of copper transport directly proportional to the concentration difference of the copper species across the solution-body interface, or (2) a facilitated diffusion process, with the rate of transport limited by the number of transport sites and the maximum velocity at which they can function. In the latter case, uptake will appear linear until a critical concentration of copper is reached where the transport system becomes saturated. Within the range of total copper concentrations used, saturation of metal uptake in brine shrimp was not observed. However, since the total concentration of copper which can be dissolved is constrained by the limited solubility of the cupric hydroxide and carbonate species, it cannot be concluded from these data whether or not the transport of copper across the solution-body interface is a simple or facilitated diffusion process.

The results concerning the effect of hydrogen ions and inorganic complexing show that the uptake of copper in brine shrimp is not simply related to the concentration of the cupric ion in the solution. Indeed, with increases of pH over the experimental range, the concentration of the cupric ion decreased sharply and yet a pronounced increase in the biological availability of copper was observed. This means that either (1) one or more of the inorganic complexes which prevail in basic conditions are available to the brine shrimp, and/ or (2) changes in the pH of the medium alter the metal uptake process, e.g. protonation of binding sites

involved in the sequestration and transportation of copper occurs. That is, the biological availability of the free cupric ion and/or the other cupric ion species taken up increases with pH. Since changes in the concentration of the cupric ion and the concentrations of the cupric hydroxide species are pH dependent, it is not experimentally possible, in an unambiguous way, to separate the effect of pH on the speciation of the metal from the effect of pH on the uptake process. Therefore, the observation that most of the variation in the availability of copper is explained by either a multiple linear or a non-linear regression model that includes the concentrations of the cupric ion and the cupric hydroxide species as independent variables, does not necessarily mean that there is also a true functional relationship between the concentration of all of these species and the availability of copper to the brine shrimp. It is, however, clear that changes in the hydrogen ion concentration have an important effect on the uptake of copper in the brine shrimp and that the observed variation in the uptake of copper is not explained by changes in the concentration of the free cupric ion alone.

The binding sites in a transport system are often weak acids, and the proportion of uncharged conjugate acid and charged conjugate base determines the number of sites available for metal binding. The proportion of conjugate base and conjugate acid is determined by the acid dissociation constant of the site. In this case, the amount of copper that is taken up is proportional to the product of the proportion of conjugate base of the site and the sum of the concentrations of the metal species taken up. Considering the cupric ion and the cupric hydroxide species as the most important biologically available species, non-linear regres-

sion analysis of the data results in a calculated pK value for the empirical binding site of 8.0.

Considering both the chemical and biological aspects of the metal uptake process, a conceptual model that relates metal speciation to metal availability can be built (Simkiss & Taylor 1989, Viarengo 1989). Changes in the speciation of a metal can have important effects on the movement of the metal in the vicinity and across the solution-body interface. Ions and other polar species in solution are not bare because of interactions with the water molecules. The strength of these interactions with the water molecules increases with the charge density of the bare species, i.e. the ratio of the charge and the size of the species. The organisation of water molecules around the species results in the formation of a solvation sphere. This is a sphere of water molecules which are closely associated to the species and move around with it as one single moving entity. The bound water molecules contribute to the size of the species and it is the number of layers of solvent molecules which determines the effective size of the solvated species. The effective size of the solvated species in turn determines the mobility of the species in the solvent and hence its diffusion rate (Burgess 1988).

Basically, the solution-body interface has the structure of a negatively charged membrane which is bounded by an electrical double layer. The membrane is a fluid mosaic of proteins in a double layer of phospholipids. Some of these proteins function as carriers that bind solute molecules and carry them across the membrane. Other proteins build channels that perforate the membrane, creating diffusion paths whose resistance is less than that of the membrane itself. The electrical double layer is a sheet of solvated anions and cations with an excess of positive charges to balance the negative surface charge of the membrane. In the bulk phase, fluid convection carries the solute species to the proximity of the interface. The solute crosses the electrical double layer by passive diffusion. The flux of a non-electrolyte is determined by the concentration gradient or chemical potential of the solute across the interface. Charged species are subject to electrical forces and the driving force for electrolyte transport is determined by the electrochemical potential gradient rather than by the chemical potential alone (Blank 1987, Cevc 1990).

Since the solution-body interface is basically a hydrophobic structure, charged and neutral polar species cannot permeate the interface by dissolving in the phospholipid bilayer membrane and diffusing across to any extent. There is an enormous interfacial resistance to the transport of polar solutes through a highly apolar phase such as the hydrocarbon core of a biological membrane. It is therefore excluded that metal uptake is

a simple diffusion process. This invokes the presence of transport systems that facilitate the translocation of the metal species across the interface (Eisenman & Horn 1983, Wills & Zweifach 1987). The strength of the interaction between a metal species and a carrier or channel binding site depends on the nature of the metal species and the binding site and is greater when the species can approach the site more closely. The results concerning the effect of inorganic complexing on the uptake of copper presented here, together with other studies concerning the effect of inorganic and organic complexing, have shown that the effect of complexation on the uptake and toxicity of copper in aquatic organisms does not depend on the stability constant or concentration product of the complexes formed (Sunda & Guillard 1976, Andrew et al. 1977, Dodge & Theis 1979, Guy & Kean 1980, Knezovich et al. 1981, Zamuda & Sunda 1982, Borgmann & Ralph 1983, Buckley 1983, Ahsanullah & Florence 1984, Zamuda et al. 1985, Blust et al. 1986, Florence & Stauber 1986, Starodub et al. 1987). This indicates that the translocation of the metal across the solution-body interface only involves weak interactions. Hence, the effect of changes in the speciation of the metal on the uptake process appears the result of (1) changes in the concentration of the copper species for which the transport system is selective by charge and/or size, and (2) changes in the selectivity and/or activity of the transport system resulting from changes in the hydrogen ion concentration.

CONCLUSIONS

The results presented have shown that changes in the chemical speciation of copper on the uptake of copper in brine shrimp are not directly related to the concentration of the cupric ion alone when changes in the concentration of the hydrogen ion are considered. The observed variations in the uptake of copper are, however, reasonably well described by either a linear or non-linear model that considers the cupric ion and the cupric hydroxide complexes as the most important biologically available species. Since the transport of the metal species across the solution-body interface requires the presence of a facilitating system, the non-linear model for copper uptake appears to be the more feasible integration of the effects of hydrogen ions and inorganic complexation on the availability of copper to the brine shrimp.

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