SUPPORTING INFORMATION FOR

Extending the biotic ligand model to account for positive and negative feedback interactions between cadmium and zinc in a freshwater alga

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Two supplementary tables

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1. Composition of culture media

Table SI.1: Total molar concentrations of constituents of low metal media used for algal growth and uptake experiments (LM) and rinse solution (LM-R) derived from the MHSM-1 medium [1]. The carbonate concentration is self-adjusted by equilibrium with the atmosphere.

Compounds	**MHSM-1**	**LM**	**LM-R**
NH_4	9.37×10^{-4}	9.37×10^{-4}	9.37×10^{-4}
Cl	5.98×10^{-6}	5×10^{-4}	5×10^{-4}
K	4.22×10^{-3}	4.72×10^{-3}	4.72×10^{-3}
PO_4	1.37×10^{-4}	1.37×10^{-4}	1.37×10^{-4}
CO_3	Atm	Atm	Atm
NO_3	5.07×10^{-3}	1.6×10^{-2}	1.6×10^{-2}
SO_4	8.12×10^{-5}	8.12×10^{-5}	8.12×10^{-5}
Mg	8.12×10^{-5}	8.12×10^{-5}	8.12×10^{-5}
Ca	6.80×10^{-5}	3.28×10^{-5}	3.28×10^{-5}
Na	1.02×10^{-4}	1.67×10^{-2}	1.67×10^{-2}
BO_3	3.01×10^{-6}	3.01×10^{-6}	
Mn	2.10×10^{-6}	3.28×10^{-8}	
EDTA	8.06×10^{-7}		
NTA		1×10^{-4}	
Fe	5.92×10^{-7}	1.28×10^{-7}	
MoO_4	3.00×10^{-8}	3.00×10^{-8}	
Zn	2.33×10^{-7}	8.61×10^{-8}	
Co	1.09×10^{-8}	6.79×10^{-8}	
Cu	7.04×10^{-8}	3.69×10^{-8}	
MOPS	1×10^{-2}	1×10^{-2}	1×10^{-2}

2. Short-term Cd uptake experiments in the presence or absence of NTA or MOPS

We tested whether 10^{-4} M NTA or 10^{-2} M MOPS (pH buffer) affected short-term Cd uptake in *C. reinhardtii*. To do so, algae (40 000 cells mL^{-1}) were exposed to the same free Cd ion concentration (10^{-9} M Cd^{2+}) for 45 minutes in four different media with or without NTA or MOPS (three replicates per media, i.e. n=3). All four media contained the major cations and anions concentrations found in the LM medium (Table SI.1) but no added essential trace elements (i.e. Fe, Cu, Co, Zn or Mn).

The first medium for our experiment was a well-buffered NTA medium (10^{-4} M total NTA; 1.84×10^{-6} M total Cd) with 10^{-2} M MOPS (i.e. the LM medium without essential trace elements); the second, a well-buffered NTA medium (10^{-4} M total NTA; 1.84×10^{-6} M total Cd) without MOPS; the third, a simplified LM-R medium (without NTA; Cd^{2+} ≈97% Cd_{total}) with 10^{-2} M MOPS; and the fourth, a simplified LM-R medium (without NTA; Cd^{2+} ≈97% Cd_{total}) without MOPS. Intracellular Cd contents were then measured by gamma spectrometry as described in the experimental section of the paper.

We found no significant differences ($p>0.05$) between intracellular cadmium quotas measured in all the different media (see Table SI.2) showing that neither 10^{-4} M total NTA nor 10^{-2} M MOPS affects Cd uptake in *C. reinhardtii* for a constant [Cd^{2+}] of 10^{-9} M. Note that the trend between the treatments was the same when normalizing the Cd uptake data with respect to cell number, surface or volume since, on a short time scale, cellular shape did not change significantly. These results indicate that, as anticipated, Cd-NTA complexes did not contribute to Cd uptake; similar results have been reported in several previous studies [2-4] . Moreover, it is shown that 10^{-2} M MOPS did not interfere with Cd uptake.

Table SI.2: Intracellular cadmium quotas (amol Cd $cell^{-1}$) measured after a 45 min exposure to 10^{-9} M Cd^{2+} in NTA-buffered media (10^{-4} M total NTA; 1.84×10^{-6} M total Cd) with or without 10^{-2} M MOPS or in LM-R media (without NTA; Cd^{2+} ≈97% Cd_{tota}) with or without 10^{-2} M MOPS. Error bars represent one standard deviation of three replicates.

	LM-R medium		NTA-buffered medium	
	Without MOPS	With MOPS	Without MOPS	With MOPS
Cd_{intra} (amol Cd $cell^{-1}$)	0.29 ± 0.026	0.25 ± 0.03	0.29 ± 0.03	0.32 ± 0.03

3. Theoretical considerations and modeling procedure: testing the assumptions of the models

We tested the assumption (in eq 3) that the term (K^{Cd}_{Cd-i} [Cd^{2+}]) in eq 3 was non-significant. Such assumption was confirmed in the case of the Cd-1 transport system (i.e. the chosen [Cd^{2+}] << $1/K^{Cd}_{Cd-1}$). However, in the case of the second Cd transport system (Cd-2), we had to choose a [Cd^{2+}] near the half-saturation constant of this transport system since the operating range of another transport system (Cd-3) was relatively close to the Cd-2 system. We thus included K^{Cd}_{Cd-2} (computed in eq 1) in eq 3. Increasing K^{Cd}_{Cd-2} up to 10^9 M^{-1} (i.e. an improbably high affinity) in eq 3 yielded a K^{C}_{Cd-2} that would still not significantly affect the modeled Cd uptake and thus K^{Cd}_{Cd-2} obtained from eq 1. The latter analysis thus proved that $K^{C}_{Cd.}$ obtained with eq 3 and K^{Cd}_{Cd-2} obtained with eq 1 are accurate parameters.

We also tested the assumption (made in equation 1) that competing ion concentrations are too low to inhibit Cd uptake. Analyses with eq 3 confirmed our assumption that the [Mg^{2+}], [Ca^{2+}], and [Zn^{2+}] used in experiments involving eq 1 were too low to significantly affect the resulting K^{Cd}_{Cd-i} and V^{Cd}_{max-1} , except in the case of Ca^{2+} and the first Cd transport system (Cd-1). In this case, we included K^{Ca}_{Cd-1} (determined with eq 3) in eq 1 to precisely determine K^{Cd}_{Cd-1} andV^{Cd}_{max-1}. The competitive effect of protons on Cd uptake was considered to be negligible at pH 7 since proton affinity constants for the Cd uptake sites were previously shown to be low at [Cd^{2+}] encompassing the operating range of the Cd-1 and Cd-2 transport systems (e.g. For [Cd^{2+}] < 4 x 10^{-4} M, K^{H}_{Cd} << 10^5 M^{-1} [5]; at 5 x 10^{-8} M Cd^{2+}, K^{H}_{Cd} = $10^{5.2}$ M^{-1} [6]). An analogous procedure was also used to model short-term Zn uptake rates and to test the underlying model assumptions.

4. Fast Zn uptake regulation and its interaction with Cd

From our competitive binding experiments, we have evidence that very fast (3-30 min) interaction between Cd/Zn and the Cd-2/Zn-2 transport system occurred at very high [Cd^{2+}] or [Zn^{2+}]. Cadmium uptake rates after a 3 min exposure were inhibited by [Zn^{2+}] greater than 5 × 10^{-8} M whereas, as measured after a 30 min exposure, Cd internalization rates tend to slightly increase (albeit not significantly; the variability among replicates at high [Zn^{2+}] was relatively high, ≈ 16-28%) in response to [Zn^{2+}] greater than 5 × 10^{-7} M and were only inhibited at high [Zn^{2+}] (≈ 10^{-5} M) (i.e., apparent K^{Zn}_{Cd-2} << 10^6) (Fig. 2B). The lower apparent Zn competitive effect for Cd uptake after a 30 min comparatively to a 3 min exposure would be consistent with the potential of an increase in [Zn^{2+}] to induce a decrease in Zn affinity of the Zn-2 uptake system without notably altering Cd binding

affinity for this system (see results determined at much lower [Zn^{2+}] and lower [Cd^{2+}] in the section "pre-acclimation to Zn or Cd and metal uptake rates"). The net result will be that Cd uptake would remain approximately stable (or decrease less easily) with increasing Zn^{2+} concentration since K_{Cd-2}^{Zn} would decrease concomitantly with an increase in [Zn^{2+}]. Another hypothesis would be that very high [Zn^{2+}] might exert a fast toxic effect on algal cells which would affect the measurements and variability of Zn/Cd uptake rates, but this explanation remains speculative.

We also noted that Zn uptake in the presence of 2×10^{-8} M Zn^{2+} could be tightly regulated over a short period of time (3-30 min) in the presence of increasing Cd^{2+} concentrations (relatively high [Cd^{2+}] > 1×10^{-8} M) ; Cd strongly competes for Zn uptake for the 3 min exposure experiment ($K_{Zn-2}^{Cd} = 10^{7.53 \pm 0.09}$) whereas after the 30 min exposure, Zn uptake rates remain constant until [Cd^{2+}] reach approximately 5×10^{-7} M (i.e. apparent $K_{Zn-2}^{Cd} = 10^{6.08 \pm 0.14}$) (Fig. 2D). We hypothesize that the surprising ability of the algal cells to tightly regulate Zn uptake rates in the presence of high Cd^{2+} might be due to the induction by high [Cd^{2+}] of an high affinity Zn transport site (with low affinity for Cd) such as the Zn-3 transport site. We already know from the data presented in this paper that Cd acclimation (albeit at lower [Cd^{2+}] and for a longer period of time, i.e. 60 h) induces an up-regulation of Zn uptake rates through the Zn-1, Zn-2 and Zn-3 transport systems (Fig. 1B). The [Zn^{2+}] (2×10^{-8} M) used here was near the saturating [Zn^{2+}] of the Zn-3 transport system (especially for algae acclimated to high [Zn^{2+}], see Fig. 1B) and since the Cd affinity is much less than the Zn affinity for the Zn-3 uptake system and that this system would be already saturated by Zn, Cd would be expected to compete with Zn for this system only at high Cd^{2+}:Zn^{2+} ratios. Using eq 3 to fit the Zn uptake rates determined after the 30 min exposure to varying [Cd^{2+}] and 2×10^{-8} M Zn^{2+} (data presented in Fig. 2D) and assuming a $K_{Zn-3}^{Zn} = 10^{10.5}$, the computed $K_{Zn-3\ 30\ min\ exposure}^{Cd}$ would be $10^{8.70 \pm 0.13}$, a value close to the $K_{Zn-3}^{Cd} = 10^{8.81 \pm 0.08}$ (determined at 1.5×10^{-11} M Zn^{2+}; see insert in Fig. 2D) reported in the present study. This calculation strengthens our interpretation that high [Cd^{2+}] rapidly affect Zn uptake rates through the Zn-2 and Zn-3 transport systems.

5. Calculations of the maximum diffusive uptake rates of Zn^{2+} or Cd^{2+}

The maximum diffusive flux of Cd^{2+} or Zn^{2+} (J , in pmol cm^{-2} s^{-1}) through the unstirred boundary layer was computed with the following equation [7]:

$$J = \frac{4\,\pi\,D\,\left(\frac{r_c r_d}{r_d - r_c}\right)\,(C_b - C_s)}{A}$$

where r_c is the radius of the cell (2×10^{-4} cm), r_d is the radius of the cell plus the thickness of the unstirred boundary layer (estimated to be 8×10^{-4} cm [8]), thus, $r_d = 1 \times 10^{-3}$ cm), *D* is the diffusion coefficient of Cd^{2+} (7.0×10^{-6} cm^2 s^{-1}) or Zn^{2+} (6.8×10^{-6} cm^2 s^{-1}) [9], A is the algal surface area (cm^2/cell), C_b and C_s are the free Cd^{2+} or Zn^{2+} concentrations in the bulk solution (in pmol cm^{-3} or nM) and at the surface (0 pmol cm^{-3} or 0 nM) respectively. The maximum diffusive fluxes were multiplied by 60 s min^{-1}, multiplied by the cellular surface area (5×10^{-7} cm^2 cell^{-1}) and then divided by the cellular volume (33.5 μm^3 cell^{-1}). The resulting limiting uptake rates of Cd^{2+} or Zn^{2+} were thus expressed in mol μm^{-3} min^{-1}.

6. References

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