Silver Interactions at Fish Gills

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Our approach is to experimentally determine metal-gill equilibrium binding constants \( (K) \) and the number of metal-gill binding sites. These values are then inserted into an aquatic chemistry program (MINEQL+; Schecher and McAvoy 1992) to predict metal-gill interactions and therefore toxicity to fish. This method has been used before to model Cu and Cd interactions at fish gills (Playle et al. 1993a, b).

Small rainbow trout \((Oncorhynchus mykiss, 1-3 \text{ g})\) were acclimated to synthetic soft water \((\text{Ca, Na } \sim 300 \mu\text{M, pH } 6.5-7.5)\), then were exposed in soft water for 2-3 h or 1 week to about 0.1 \( \mu\text{M Ag} \) \((\sim 11 \mu\text{g-L}^{-1})\). Water chemistry of the soft water was varied by adding Ca, Na, or H\(^+\) (for competition experiments), or thiosulphate \((S_2O_3^{2-})\), dissolved organic carbon (DOC), or Cl (for complexation experiments). Fish gills were removed at the end of the experiments, and gill Ag was measured by graphite furnace atomic absorption spectrophotometry. Silver concentrations of 0.1 \( \mu\text{M} \) were used because this concentration yields measurable Ag accumulation on the gills, yet was not toxic in our experiments. The 96 h LC50 for Ag in soft water is between 0.06 and 0.15 \( \mu\text{M} \) (Davies et al. 1978; LeBlanc et al. 1984).

About 30X more thiosulphate than Ag was needed to prevent Ag deposition on trout gills. This protective effect of Ag persisted for 6 d. From the thiosulphate data, the conditional equilibrium constant of Ag-gill interactions must be greater than that for Ag-thiosulphate. That is, \( \log K_{Ag-gillAg} > \log K_{Ag-S2O3} = 8.8 \). We calculated that \( \log K_{Ag-gillAg} = 10.0 \), with approximately 1.3 nmole Ag binding sites per fish. Inserting these values into the MINEQL+ program gave a good fit between observed and predicted gill Ag concentrations \((r=0.924 \ (6) ; p<0.01)\). In contrast, gill Ag concentrations predicted on the basis of free Ag\(^+\) concentrations calculated by MINEQL+ gave a bad fit between observed and predicted gill Ag \((r=0.525 \ (6) ; p>0.05)\).

At high enough concentrations \((11.3 \text{ mM})\), Cl was able to keep 0.1 \( \mu\text{M Ag}\) off trout gills by complexing the Ag as AgCl and AgCl\(^-\). Similarly, 24 mg C-L\(^-1\) DOC kept Ag off the gills. From these data, the binding constant between Ag and DOC can be calculated at \( \log K_{Ag-DOC} = 9.0-9.2 \), and \( \log K_{Ag-Cl(n)} = 4.8-5.7 \). There are no values in MINEQL+ for Ag-DOC interactions, but \( \log K_{Ag-Cl(2)} = 5.3 \), right in the middle of the range calculated from our experimental data.

As for competition for Ag binding sites on the gills, 16 mM Na kept Ag off the gills, pH as low as pH 4.5 did not, and concentrations of Ca up to 10.6 mM
also did not keep Ag off the gills. From these results, \( \log K_{\text{Na-gillAg}} = 4.7-5.7 \), \( \log K_{\text{H-gillAg}} < 7.1 \), and \( \log K_{\text{Ca-gillAg}} < 4.5 \).

Calculated log \( K \) values were optimized to the experimental data, and the final log \( K \) values were inserted into the MINEQL\(^+\) program. The model was tested using fish exposed to Ag in natural waters. Observed and model predicted gill Ag concentrations agreed remarkably well, except for one case. This exception was City of Waterloo tapwater, for which the model predicted background gill Ag concentrations but we observed high gill Ag. Waterloo tapwater has very high Ca concentrations (~3,000 \( \mu \text{M} \)); the model predicted low gill Ag mostly on the basis of these high Ca concentrations. This exception to the otherwise good predictive capabilities of the model may indicate a kinetic constraint on the thermodynamic basis of the model.

Silver accumulation on fish gills is exceptionally fast, so that cold trout (with low metabolic rate; 4\(^\circ\)C) end up with as much Ag on their gills in 2.5 h as do warm trout (with higher metabolic rate; 20\(^\circ\)C), even though warm trout initially accumulated much more Ag on the gills. Warm fish (18\(^\circ\)C) given twice as much oxygen need to ventilate half as much water to satisfy their doubled oxygen demand, so accumulated only as much Ag on their gills as did fish held at 8\(^\circ\)C. Finally, cold trout (8\(^\circ\)C) given half as much oxygen need to breathe twice as much water to satisfy their oxygen demand, so accumulated as much Ag on the gills as trout held at 18\(^\circ\)C. These results indicate, at least for initial accumulation of Ag on the gills, that gill Ag concentrations are dependent on the "dose" of Ag reaching the gills.

References


Questions & Answers: Silver Interactions at Fish Gills

Q. RUSSELL ERICKSON (EPA-Duluth): Your data on the chloride addition suggests that there was no real effect, no competition for uptake — the way you were measuring gill uptake for silver, at 0.3 and 0.4 millimolar — were your concentrations correct?

A. Right.

Q. OK, it was taking up as much silver as in the absence of chloride. Yet Chris' data show a major effect on toxicity at 0.1. Would you comment on what implication that has to do with how your uptake at the gill is, in fact, correlating to the toxic mechanism, or if it isn't?

A. It's kind of difficult to compare studies in that way. Those were our baseline conditions of 0.3, 0.4. If in Chris' lab they could get chloride down much lower, then that's great. I would predict that if we had run our experiment at lower chloride concentrations, we would have ended up with more silver on the gills. So, I guess that's the answer — sort of a different starting point.

Q. JIM KRAMER (McMaster Univ.): I want to go back to your model and emphasize, or look at, the silver/DOC stability constant, which you gave a log value of nine, which, to me, would be very high unless you had some sulfur binding there. Can you tell me a little bit about your black box DOC?

A. I can tell you a little bit, not a lot about it. I got my dissolved organic carbon from Ken Burnison. Ken's at the Centre for Inland Waters and he gets it from Luther Marsh, up near Orangeville. He's done some characterization of it, but I really can't comment on that.

Q. (From Audience): How do you get your stability constant?

A. Oh, yes, it's a bit of a guessestimate. We make an estimate of the number of binding sites per milligram of carbon per liter, and then make an estimate of the strength of binding. You can have more binding sites and lower binding constant, or fewer binding sites and a higher constant.

Q. (Audience): What you know is the concentration.

A. Yes. I don't think it's particularly out of line for anything I've seen.

Q. KRAMER: Well the only thing we find in the literature is . . .

A. . . . is for copper.

Q. . . . is sulfur driven. That's what I'm saying.

A. On the organic carbon?

Q. Yes, on the organic carbon.

A. Sure.

Q. We can use DOS.
A. Yes, DOS. We're working on it.

Q. GREGORY CUTTER (Old Dominion Univ.): You had a number for the apparent stability constant for the gill site. Do you want to speculate on what it is, then?

A. Well, based on Chris Wood's data, I'd say it's some kind of protein involved in sodium or chloride uptake. But in my model, it doesn't really matter what it is. It's just a site. But, again, based on Chris' data, I'd say it's some kind of active ion uptake site.

Q. I guess I was thinking more of a chemical . . .

A. Oh, I see what you mean.

Q. I think you have to have a thiol.

A. Yes, well it would be — I think it would be a carboxyl group. Proteins have lots of carboxyl groups on there.

Q. KRAMER: We run them the same way though. We don't get numbers like nine for carboxyl. Not unless you've got some exotic chemical that nobody knows about.

A. Oh, okay. Yes, there's sulphydryl groups, too. Sure.

Q. (Audience): All I'm saying is, if we get the exponent wrong, we won't get these.

A. No. Again, I'm not really speculating. Carboxyl groups could be sulphydryl groups. I think the value is reasonable.

Q. (Audience): Yes, I don't have a question on the value. I just think, what do you speculate? Because that, mechanistically, is what you need to know.

A. Yes.