Positive Pressure Effect on Manganese Binding by Bacteria in Deep-Sea Hydrothermal Plumes

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A positive pressure effect (1.4 to 3.3 x) on the binding of Mn\(^{2+}\) by a natural population of bacteria in a deep-sea hydrothermal plume was discovered over the intermediate pressure range of 1 to 200 atm (1 to 200 bars; ca. 1.01 x 10\(^2\) to 2.03 x 10\(^4\) kPa). The data suggest Mn\(^{2+}\) binding is functionally barophilic rather than simply barotolerant.

Microorganisms play a dynamic role in the marine geochemical cycling of manganese. Manganese is important both as an essential micronutrient (17) and as a valuable tracer of geochemical processes, such as deep-sea hydrothermal vent emissions (1, 2, 14) or offshore advection of waters impacted by the redox chemistry of continental slope sediments (15). Microbial activity can greatly increase the rate of Mn(II) removal onto particles throughout the water column over that resulting from abiotic processes (3, 22). These microbial Mn oxidation rates can be significantly inhibited by light, reduced oxygen partial pressures, or excessively low or high temperatures (22). Erlich (9) reported on several cultures isolated from deep-sea ferromanganese nodules that were capable of oxidizing Mn(II) over wide pressure ranges (1 to 476 atm [ca. 1.01 x 10\(^2\) to 4.82 x 10\(^4\) kPa]). However, despite growing evidence suggesting that barophilic bacteria are widespread and may even functionally dominate in deep-sea environments (5, 6, 16), the influence of pressure on the binding and oxidation of Mn by natural populations of bacteria has not been reported. The effects of pressure on Mn-binding rates were specifically addressed during a recent field study of microbial Mn removal in extended deep-sea hydrothermal vent plumes (Fig. 1). The experimental results indicate that removal of Mn from the plume is largely a barophilic microbial process.

Hydrothermal plumes were tracked in real time with a sensitive electronics package deployed from the surface ship (1, 3). The plume was thus selectively and remotely sampled with clean 30-1 Niskin (General Oceanics) bottles (3). At the surface, water was quickly transferred to ultraclean Teflon or polycarbonate bottles (3). The bottles were spiked with \(^{54}\)Mn\(^{2+}\) and poisoned as appropriate (sodium azide, 15 mM final concentration [19]). Bottles intended for in situ incubations were then quickly secured to the hydrowire (for 6- to 20- h incubation) or a bottom-anchored mooring (for 21-day incubations) immediately prior to deployment. Once deployed, usually within 1 h (range, 0.25 to 1.5 h), the bottles were kept near the depth of their original collection (~2,000 m) for the duration of the incubation period. Control incubation bottles were replaced in the cold baths on board ship and kept in the dark at 1 atm and 15 to 2°C (original in situ temperature of the plume). The experiments were terminated by filtering 0.5 to 1.0 liter through a membrane filter (pore size, 0.2 \(\mu\)m; Millipore Corp.). Gamma radiation from the \(^{54}\)Mn in the sample was counted directly with a NaI crystal well detector. Data were corrected for radioactive decay and filter blanks. Carrier-free, high-specific-activity tracers were used; approximately 1 pM Mn was added to the samples, which contained on the order of 1 to 100 nM natural Mn.

The general hypothesis that microbial activity significantly influences the Mn-binding process was reaffirmed. The total (unpoisoned) binding rate was always significantly greater than the rates derived from samples treated with sodium azide (Table 1). Sodium azide is a metabolic poison which has been shown to not interfere with Mn chemistry at the concentrations and incubation times used here (19, 22). Results of the present study further allow a distinction of biological Mn removal on the basis of incubation pressure. Subsamples incubated in situ (~200 atm [ca. 2.03 x 10\(^4\) kPa]) consistently bound more \(^{54}\)Mn than did control samples incubated at 1 atm on board ship (Table 1). The samples were collected within the plume maximum along a constant pycnocline at increasing distance from a known active vent field. The only time course data available which appraise the effects of ambient deep-sea pressure versus atmospheric sea surface pressure on the rate of manganese binding onto particles are shown in Fig. 2. At 15 h the in situ \(^{54}\)Mn-binding rates were the same or slightly higher than the 1-atm rates for the 2,052- and 2,077-m samples, respectively. At 505 h, however, samples from both depths showed overall in situ binding rates considerably greater than the 1-atm rates. While the overall rate of \(^{54}\)Mn binding at in situ high pressures increased nearly threefold in both samples from 15 to 505 h, the atmospheric pressure rates decreased by a factor of 3 or more.

Additionally, it is significant that the relative biological contribution to the binding rate also greatly increased with time in the in situ-incubated samples. Poisoned treatments at 1 atm were inadvertently omitted from the experiment for which results are shown in Fig. 2. However, the results of a separate 48-h time series at 1 atm are shown in Fig. 3; both the total binding rate and the total-minus-poisoned rate decreased over the 48-h incubation period. However, the data in Table 1 indicate that the inhibition from azide poisoning was greater at 1 atm than at 200 atm suggesting that sodium azide only partially inhibits the biological binding of Mn at 200 atm and is more effective at only 1 atm, perhaps due to a negative synergy with decompression stress. This would further suggest that the total-minus-poisoned fraction underestimates the biologically mediated contribution to the total \(^{54}\)Mn binding at 200 atm.

The results discussed here are suggestive of a system adapted to elevated pressures (18, 20). Destabilization of a critical protein at reduced pressure, for example, could inhibit enzyme-catalyzed Mn(II) oxidation; inhibition of the
The increased inhibition of Mn deposition associated with the bacterial polymer capsules found in abundance at plume depths (3, 5; unpublished data) was observed with a prolonged incubation at 1 atm. An alternative explanation for the present results provides for a binding mechanism involving predominantly organic polymers produced by barophilic bacteria. In strong support of this scenario is the finding that a majority of the particulate Mn in extended plumes is contributed by the Mn deposits associated with the bacterial polymer capsules. The increased inhibition of Mn binding by poison at 1 atm relative to that at 200 atm may occur via negative synergy acting on the production of key polymer material. The exacerbated effect of prolonged decompression may be a function of depressed production of new polymer, whereas the stimulatory effect of a reacclimation period following recompression (Fig. 2) could result from the recovered production of new polymer.

The degree of recovery from decompression stress during long-term incubation under recompressed conditions may be related to the duration of the decompression period. Al-
though most reports show no irreversible damage to measured cell functions as a result of brief decompression periods when the ambient cold temperature is maintained (11, 12, 25), severe sensitivity to a 5-h decompression at room temperature has been reported for known psychrophilic barophiles (23). Following recompression, on the other hand, recovery of original in situ rates may depend on the reacclimatization of the responsible bacterial population and subsequent recovery of the affected systems.

Thus, the positive influence of in situ incubations on Mn-binding rates can best be attributed to the positive effects of increased pressure on microbial activity. The response is at least functionally barophilic with respect to Mn(II) binding and presumably oxidation. Simple barotolerance is not indicated since the binding rates are inhibited at sea surface pressures, severely so over long periods. These data were derived from studies of natural populations under nearly in situ conditions. The relationship of pressure to population growth, as typically measured by its effect on generation time or change in cell number or substrate utilization, would not necessarily correspond to Mn-binding activity. Hence, it may be useful to distinguish between the involvement of true barophiles (26), organisms that grow optimally at elevated pressures, and those that exhibit one or more functionally barophilic responses exclusive of, or at least of unknown relation to, actual growth requirements. However, this exercise is greatly complicated by the dependence of demonstrated barophilic growth on substrate choice and concentration (5).

The discovery of a positive pressure effect on the binding of Mn by bacteria in deep-sea hydrothermal plumes has considerable bearing on the manner in which radotracer experiments to determine removal rates and residence times of dissolved Mn are conducted. Moreover, extrapolation of the results to the deep sea in general may be plausible since all samples were collected from the extended plume, generally several kilometers from the active vents themselves. The effluent hydrothermal solutions are highly diluted and therefore detectable only by means of a few sensitive chemical tracers such as manganese. Additional experiments with in situ incubations and recompressed samples from within and outside of a hydrothermal plume are needed to provide a basis for quantitative estimates of microbial and total Mn-binding rates as they occur in situ.

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