Studies of the physiology of chemosynthetic fauna at Cold Seeps in Sagami Bay, Japan

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Comparisons of cold seep habitats in Sagami Bay, Japan, and Monterey Bay, California, U. S. A. have included studies of the geology and geochemistry of seeps as well as the biology and ecology of seep-associated fauna. In this paper we present results of research concerning the physiology of chemosynthetic fauna found at cold seeps in Sagami Bay.

Haemolymph sulfide binding properties were measured for individuals of *Bathymodiolus platifrons*, *Calyptogena soyoar*, and an undescribed vestimentiferan worm (*Lamellibrachia* sp.) that inhabit seeps in Sagami Bay. *C. soyoae* and *Lamellibrachia* sp. haemolymph was shown to bind sulfide more than 10 times ambient levels. Haemolymph from *B. platifrons* did not show evidence of sulfide binding at any level of ambient sulfide concentrations.

Key words : Cold seep community, Physiology, Ecology, Sagami Bay, Monterey Bay

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1. Introduction

Chemosynthetic communities associated with seepage of sulfide or methane-rich fluids have been found in various settings since their discovery in the late 1970s and early 1980s (Lonsdale, 1979 ; Paull *et al.*, 1984 ; Kennicutt *et al.*, 1985) and appear to be common along continental margins (Barry *et al.*, in press a). Chemosynthetic communities dominated by vesicomyid clams, mytilid mussels, vestimentiferan or pogonophoran worms, or free-living bacteria, have been reported from hydrocarbon seeps (Kennicutt *et al.*, 1985, 1988, 1989), groundwater seeps (Paull *et al.*, 1984 ; Hecker, 1985), methane seeps (kennicutt *et al.*, 1985 ; Dando *et al.*, 1991), accretionary prisms assicuated with subduction zones, other sites of tectonically-compressed sediment (Suess *et al.*, 1985 ; Laubier *et al.*, 1986 ; Boulègue *et al.*, 1987 ; Cadet *et al.*, 1987 ; Le Pichon *et al.*, 1987 ; Ohta and Laubier, 1987 ; Hashimoto *et al.*, 1987, 1989 ; Jollivet *et al.*, 1990 ; Barry *et al.*, in press a), and relict organic material buries in debris flow deposits (Embley *et al.*, 1990). Fossil cold seeps and associated faunal assemblages have also been reported from several sites (Beauchamp *et al.*, 1989 ; von Bitter *et al.*, 1990, 1992 ; Campbell and Bottjer, 1993 ; Aharon, 1994 ; Clari *et al.*, 1994).

During the past few years, comparisons of cold seep communities in Sagami Bay, Japan, and Monterey Bay, California, U. S. A. have been performed by scientists from JAMSTEC, Monterey Bay Aquarium Research Institute (MBARI), and Moss Landing Marine Laboratories (MLML). Dives at cold seeps on either side of the Pacific have been made using manned submersibles ("Shinkai 2000") and ROVs ("Dolphin 3K", Ventana), to support studies of biology, geology and geochemistry.

Similarities between the geology and geochemistry of Sagami and Monterey Bay seeps (Greene *et al.*, ms this issue), appear responsible for partial overlap in the composition of chemosynthetic fauna. Vesicomyid clams are the dominant metazoan fauna of cold seeps in both Sagami Bay (Hashimoto *et al.*, 1989) and Monterey Bay (Barry *et al.*, in press a). However, studies of the fluid chemistry in both areas show that although there is overlap among sulfide concentrations within aggregations of vesicomyid clams on either side of the Pacific (Hashimoto *et al.*, 1995 : Barry *et al.*, in press b), the levels of sulfide and methane vary considerably among sites.

Relatively little information has been published concerning the biology of vesicomyids from cold seep habitats. Most information concerning vesicomyid biology has come from studies of *Calyptogena magnifica*, the very large species common at hydrothermal vents (Boss and Turner, 1980 ; see Childress and Fisher, 1992).

Research described in this paper concerns the sulfide-binging affinity of blood serum from *Calyptogena soyoae*, mytilid mussels (*Bathymodiolus platifrons*), and an underscribed vestimentiferan worm (*Lamellibrachia* sp.). Results from these studies in Sagami Bay and similar studies of cold seep vesicomyids from Monterey Bay (and hydrothermal vent sites), represent the first such comparisons of the physiology of vesicomyid clams among seep and vent sites.

2. Method

2.1 Study Site

Hashimoto *et al.* (1989) present a thorough description of cold seeps in Sagami Bay and the biota inhabiting these seeps. Geochemistry of pore waters was examined by Masuzawa *et al.* (1995), and the relation of pore fluid chemistry to the distribution of *Calyptogena soyoae* populations at the Hatsushima site cold seeps was discussed by Hashimoto *et al.* (1995). Additional information on the existence of thermal anomalies related to volcanogenic heating is presented in Naka *et al.* (1991).

2.2 Haemolymph Sulfide Binding

Sulfide binding studies were carried out on haemolymph of vesicomyid clams (*Calyptogena soyoae* ; collected on "Shinkai 2000" dives 828 and 829), mytilid mussels (*Bathymodiolus platifrons* ; collected on dives 829 and 831), and vestimentiferan tube worms (*Lamellibrachia* sp. ; collected on dive 832). Haemolymph samples were removed from specimens immediately following recovery of the submersible. A 0.5m*l* aliquot of haemolymph was immediately placed in a vial containing 0.5 m*l* fixing solution (1 M zinc acetate) following dissection and exsanguination of each specimen. Additional 0.5 m*l* aliquots were injected into dialysis cassettes (Pierce, 10k MWCO Slide-A-Lyzer), which were distributed among dialysate solutions. Sulfide stock solution was prepared by dissolving sodium sulfide crystals in nitrogen-bubbled (oxygen-free) sea water, and adjusting the pH with HCl to 8. Sulfide stock solution was serially diluted with nitrogen-bubbled sea water to produce sulfide dialysate solutions of 0.05, 0.10, 0.25, 0.50, and 1.00mM H_2S. Haemolymph samples were dialyzed for 24 hours at 4℃, extracted from dialysis cassettes, and fixed as above.

Fixed haemolymph samples were stored at room temperature for several weeks, then analyzed for sulfide content using a protocol modified from cline (1969). Briefly, 100μl of fixed sample was combined with 900 μl distilled, deionized water, 20μl DPP solution (0.48 g N,N-dimethyl-p-phenylene diamine dihydrochloride in 100ml 6M HCl) and 20μl ferric chlorife solution (1.6 g $FeCl_3$ in 100ml 6M HCl). After 60 min., samples were spun for 5 min. at low speed to remove large particles, and the absorbance of the supernatant was measured at 670 nm on a UV-vis diode-array spectrophotometer (HP 8452A). Sulfide concentrations in the fixed haemolymph samples were calculated from a standard curve, based on solutions of known sulfide concentration processed simultaneously with haemolymph samples.

3. Result

3.1 Haemolymph Sulfide Binding

Sulfide concentrations in haemolymph from freshly-collected *Calyptogena soyoae* ranged from 0.1-0.4mM, with a mean of 0.21mM (n=7). Sulfide in the lamellibrachiid worm was comparable, near 0.25mM. *Bathymodiolus platifrons* had less sulfide in the haemolymph (0.001, 0.016, and 0.049mM in three individuals tested).

Sulfide binding experiments demonstrate that *Calyptogena soyoae* and *Lamellibrachia* sp. contain haemolymph which binds sulfide at high affinity, whereas haemolymph of *Bathymodiolus platifrons* exhibited no such affinity (Fig. 1). Haemolymph of *C. soyoae* showed the greatest affinity for sulfide among these species, with 1.5±0.5(s.d., n=7)mM bound at 0.1 to 0.25 mM H_2S, or a 6 to 8-fold concentration factor. At lower ambient sulfide levels (0.05mM H_2S), haemolymph concentrations also decreased, but were 20-fold greater than environmental levels. The affinity of lamellibrachiid haemo-

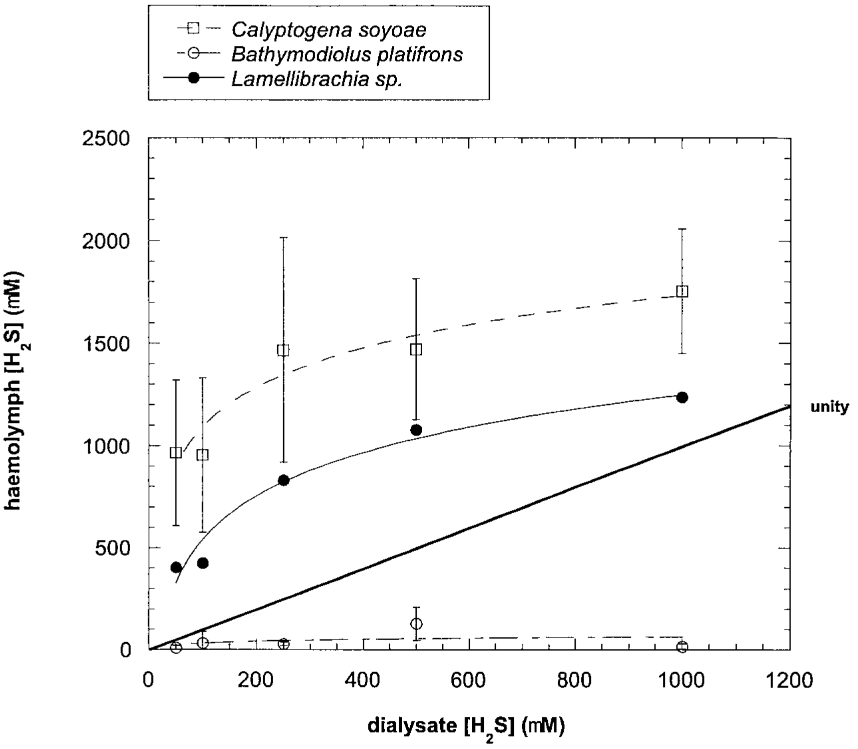

Fig. 1 Plot of haemolymph binding affinity from *Calyptogena soyoae*, *Bathymodiolus platifrons*, and an undescribed species of *Lamellibrachia*, from the Hatsushima cold seep site in Sagami Bay, Japan. Data represent sulfide concentrations in dialysed blood samples incubated for 24h at specified ambient sulfide levels.

lymph was slightly lower, with 0.83 mM bound at 0.25 mM H_2S (n=1), and a oncentration factor less than 10 for all dialysate levels. In both species, H_2S in haemolymph appeared to approach an asymptotic limit with increasing dialysate H_2S concentration suggesting saturation of the binging moiety at external concentrations well above 1 mM H_2S. Linear regressions performed on double-reciprocal plots of these data suggest that haemolymph approaches saturation in *C. soyoae* near 2 mM H_2 S, and 1.5 mM H_2S for *Lamellibrachia* sp. Sulfide levels in *Bathymodiolus platifrons* haemolymph were lower than dialysate concentrations in all trials.

4. Discussion

4.1 Role of Haemolymph Sulfide Binding Affinity

The ability of chemosynthetic species to bind sulfide very likely influences both their distribution among sites and their metabolic potential. Because delivery of sulfide from the seep pore waters to intracellular bacterial symbionts is facilitated by haemolymph, the capacity of haemolymph to bind sulfide is key in relating environmental chemistry to organism function (e.g., metabolism and growth). In vesicomyid clams and vestimentiferan worms, sulfide binding ability plays three potential roles. First, it allows the host to concentrate sulfide, thereby providing bacterial symbionts with intracellular sulfide levels that are considerably higher than exist externally. Second, it protects the host from the toxic effects of sulfide by sequestering sulfide from the host tissue in a "bound" form. Third, it provides a sulfide-rich reservoir that can be utilized by the symbionts during periods of low environmental sulfide availability.

Patterns of sulfide binding affinity and species-habiat associations observed for vesicomyid bivalves is Monterey Bay indicate that binding affinity is highest for species inhabiting low sulfide habitats, and vice versa. *Calyptogena pacifica* is most abundant at seeps with low interstitial sulfide levels (<0.1mM H_2S ; Barry *et al.* in press b), and can concentrate sulfide in haemolymph well over 10X ambient levels, and perhaps over 100X ambient concentrations. In contrast, *C. kilmeri*, which thrives in seeps with sulfide levels up to 100 times higher (>5 mM H_2S) than sites inhabited by *C. pacifica*, has a much lower binding affinity for sulfide (Kochevar *et al.*, ms).

Calyptogena soyoae inhabits seeps in Sagami Bay with sulfide concentrations near 0.5mM H_2S in the top 20 cm of sediment (Hashimoto *et al.* 1995). This level is intermediate between the low sulfide levels (<0.1 mM H_2S) typical of seeps in Monterey Bay dominated by *C. pacifica*, and the high sulfide seeps (>5 mM H_2S) in which *C. kilmeri* dominates. Based on these ambient pore fluid sulfide levels, we expected the haemolymph binding affinity of *C. soyoae* to be intermediate between the two Monterey vesicomyids. Surprisingly, the sulfide binding affinity of haemolymph from *C. soyoae* is unexpectedly high, and even exceeds *C. pacifica* at intermediate levels of dialysate.

Assuming that binding affinity for sulfide at least partially determines the levels of sulfide available to bacterial symbionts for vesicomyids, several potential explanations can be proposed for the divergent pattern of sulfide binding and habitat association among vesicomyids from Monterey and Sagami Bay cold seeps. First, symbionts are known to differe among species (Distal *et al.*, 1994), and may have vastly differing requirements for sulfide, thereby decoupling patterns of sulfide binding and habitat sulfide levels among species. Presently, we have no data to evaluate this hypothesis. Second, although sulfide binding affinity may partially determine habitat tolerance among species (e.g. *C. pacifica* and *C. soyoae* tolerate low sulfide environments), differences in binding affinity may be related to the combined effects of habitat tolerance and metabolic demand. Thus, high binding affinity may enable species increase the rate of sulfide transport to symbionts, as well as maintaining high haemolymph sulfide levels, thereby supporting higher metabolic activity (e.g. higher growth ; see below). Finally, high binding affinity may result from a combination of factors, including the ability to tolerate spatial or temporal changes in sulfide concentration, or both. Thus, environmental sulfide levels near *C. soyoae* may be more temporally variable than in Monterey Bay, requiring a high binding affinity to support symbionts during periods of low environmental sulfide availability, but also a high capacity for tolerance during periods of high sulfide levels.

This last hypothesis is supported by our finding of low sulfide in haemolymph from freshly-collected *C. soyoae*, suggesting that environmental levels may be low. Analyses of haemolymph from freshly-collected vesicomyid clams and lamellibrachiid worms suggest that sulfide levels to which these animals are exposed are low-on the order of 0.1 mM H_2S. These findings are consistent with measurements of very low sulfide concentrations in haemolymph of freshly-collected *Bathymodiolus platifrons*. Congeneric mytilids found in hydrothermal vents and hydrocarbon seep settings, harboring either thiotrophic (*Bathymodiolus thermophilus*) or methanotrophic (Seep Mytilid Ia) symbionts, have not been found to concentrate sulfide from the external environment (Childress and Fisher, 1992; Kochevar *et al.*, in prep); thus their haemolymph sulfide concentrations approximate *in situ* sulfide levels.

4.2 Physiological Ecology of Thiotrophic Metazoan Symbioses

The combined effects of haemolymph sulfide binding affinity, symbiont sulfide requirements, and interstitial sulfide levels appear to play a large role in the ecological distribution of chemosynthetic species. High haemolymph sulfide affinity allows *Calyptogena pacifica* to inhabit seeps with very low environmental sulfide levels, presumably by enabling this species to elevate intracellular sulfide levels well above external levels at the point of delivery (i.e., the symbionts). Furthermore, the apparent de-coupling of carbon uptake from sulfide uptake (Kochevar *et al.*, in prep.) suggests that the haemolymph also serves an important reservoir function, buffering symbionts from fluctuations in external sulfide levels. However, the high affinity of *C. pacifica* haemolymph also results in saturation of the binding moiety at relatively low external sulfide levels, making this species vulnerable to sulfide toxicity in high sulfide habitats. *C. kilmeri*, on the other hand, exhibits low haemolymph sulfide affinity. This may reflect a relaxation of the need to maintain a reservoir of sulfide for the symbionts, suggesting a steady supply of sulfide in the environment; and may also provide a higher level of sulfide tolerance than that of *C. pacifica*, thus allowing it to thrive in habitats with much higher sulfide levels. Thus, there appear to be significant physiological trade-offs between sulfide tolerance and energetic uptake (i.e. bacterial productivity).

Because vesicomyid clams are found intimately associated with sulfide-rich substrate, they have often been characterized as "bridging the gap" between reducing and oxidizing conditions in a spatial sense. This is in contrast to vestimentiferan worms, which are adapted for exposure to oxidizing and reducing conditions which alternate temporally on very rapid time scales. However, as our knowledge about vesicomyids increases, particularly in the context of cold seep habitats, it is clear that physiological adaptations, as well as species distribution, are likely the product of both spatial and temporal variability in sulfide availability. Furthermore, the co-occurrence of vesicomyid clams, vestimentiferan worms, and mytilid mussels at Sagami Bay cold seeps provides a unique model system in which to refine our understanding of the functioning of these three groups of organisms.

Studies of Monterey Bay and Sagami Bay cold seeps to date have provided much useful information about water chemistry, animal physiology, and community structure. The striking similarities among the various cold seep settings suggest that ongoing comparative studies will help lead to a broader understanding of these habitats worldwide. To better understand Monterey Bay and Sagami Bay cold seep communities, and to facilitate comparisons among them, we must begin to integrate detailed singletime-point observations with longer-term measurements which will provide a temporal context in which to understand patterns of change in water chemistry and animal distribution. Furthermore, ongoing developments at MBARI and JAMSTEC in *in situ* monitoring technology hold great promise for furthering our understanding the physical and chemical processes which underlie observed patterns in physiology and ecology of chemosynthetic fauna.

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