Life in Sulfidic Environments: Historical Perspective and Current Research Trends

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SYNOPSIS. The discovery of the deep-sea hydrothermal vents and their associated fauna in 1977 was a watershed for the recent interest in the biology of sulfidic environments. From the initial questions concerning how organisms survived at the high sulfide concentrations around the vents, research quickly focused on the previously unrecognized sulfur-oxidizing chemoautotrophic invertebrate/bacterial symbioses whose nature was discovered in 1980. There followed a successful and frenzied search for other reducing habitats harboring non-vent chemoautotrophic symbioses. The interest in the biology of species living in sulfidic habitats has since expanded to include the non-symbiotic species, species with sulfur-oxidizing symbionts and species with methanotrophic symbionts, all of which must be adapted to tolerate sulfide and many of which are adapted to utilize sulfide in various ways.

INTRODUCTION

Sulfur is an abundant and widely distributed element on the face of the earth (Nriagu and Hem, 1978). It is essential to living organisms as a component of some amino acids and other chemical structures. In the form of sulfate it is a major ion in saltwater. As the smelly gas hydrogen sulfide it is frequently produced where organic matter is being broken down by anaerobic bacteria. Gaseous oxides of sulfur are major components of manmade air pollution. These are only a few examples of the occurrence of this ubiquitous element. The gas hydrogen sulfide and its ionized forms HS⁻ and S²⁻ are pervasive in the biosphere, being found as products of anaerobic metabolism in aquatic environments and animal guts, as well as by-products of metabolic processes within organisms. Although the potential toxicity of sulfide is quite high (Smith et al., 1979), its effects in natural settings have been relatively poorly studied in the past.

This relative lack of study is probably the result of the difficulty in working with sulfide. It has a fairly complex chemistry, is highly reactive and spontaneously oxidizes in the presence of oxygen (Chen and Morris, 1972; Millero et al., 1987; Nriagu and Hem, 1978; Smith et al., 1979). These characteristics make it rather difficult to establish experimental conditions of known sulfide concentrations in the presence of oxygen or metals.

Early biological work was concerned primarily with toxicity for humans and other higher vertebrates in industrial settings (Smith et al., 1979; Smith and Gosselin, 1979) and with basic biochemical questions (Keilin, 1929). Another major area of concern was the use of sulfide as a reductant by chemosynthetic bacteria (Brock and Schlegel, 1989; Kelly, 1982; Zavazarin, 1989). Work was also done on the effects of sulfide on the distribution and physiology of intertidal animals (Groenendaal, 1979; Schick, 1976; Theede, 1973; von Oertzen, 1973), and the study of the “sulfide-system,” an interstitial biotic community beneath the oxidized layer in marine sand bottoms (Fenchel and Reidl, 1970; Powell et al., 1979; Powell et al., 1980).

DISCOVERY OF THE HYDROTHERMAL VENTS AND CHEMOAUTOTROPHIC SYMBIOSES

In 1977, the astonishing discovery of rich biotic communities around deep-sea hydro-
thermal vents on the Galapagos Rift in the Eastern Pacific attracted the attention of biologists and the media. This discovery was unprecedented and totally unexpected. Geologists using the submersible Alvin were seeking hydrothermal springs on the mid-ocean ridges (Corliss and Ballard, 1977; Corliss et al., 1979). Although they did find those, the major excitement concerned their finding of dense communities of large, colorful, and unusual animals living around the hot springs. Among the most obvious questions about the vent communities was the food source which supported them and hypotheses were put forward which explained how they could be supported by surface production (Enright et al., 1981; Lonsdale, 1977).

The first biological expedition to study the deep-sea hydrothermal vents took place in 1979 with J. F. Grassle as chief scientist. In the same year, geologists found similar vents and communities at sites far removed along the East Pacific Rise suggesting that deep-sea vents and their biological communities were a worldwide phenomenon (Spiess et al., 1980). Results from this first biological expedition described the distribution of animals around the vents and made clear the exposure of vent species to the warm, sulfide-rich vent waters (Hessler and Smithey, 1983). Stable C isotope analyses suggested a novel, non-photosynthetic food source for the vent mussels (Rau and Hedges, 1979). However, the revolutionary discovery was that endosymbiotic sulfur-oxidizing chemoautotrophic bacteria were living within the tissues of the major sessile species of the vent community (Cavanaugh et al., 1981; Felbeck, 1981; Felbeck and Somero, 1982). This type of symbiosis was unprecedented and unexpected. Although free-living sulfur-oxidizing chemosynthetic bacteria were well known, there had never been a suggestion that they might form symbioses with invertebrates.

**The Search for More Symbioses**

Following this initial discovery, there was an immediate, frenzied search for more such symbioses in other reducing habitats. Early discoveries included the gutless or nearly gutless bivalves of the genus *Solemya* (Cavanaugh, 1980; Felbeck et al., 1981) living in reducing muds in eelgrass beds or in a sewage sludge outfall and having sulfur-oxidizing chemoautotrophic bacteria in their gills. Lucinid clams living either in organic-rich deep-sea sediments or intertidal sediments were next found to harbor such symbionts in their gills (Felbeck et al., 1981; Fisher and Hand, 1984). Vesicomyid clams from habitats other than the vents were soon discovered to have chemoautotrophic symbionts (Cavanaugh, 1983; Childress and Mickel, 1982; Felbeck et al., 1981). A gutless, interstitial tubificid was also found to have sulfur-oxidizing chemoautotrophic symbionts (Giere, 1981; Giere et al., 1984). The phylum Pogonophora, long a mystery due to its lack of a gut, was re-examined and chemoautotrophic bacteria were found in its trunk (Southward et al., 1981).

During this same time period, increased exploration by geologists revealed the presence of hydrothermal vents and vent communities associated with the mid-ocean ridges as well as with subduction zones all over the globe (Tunnicliffe, 1991). Given the example of the vent faunas, other deep-sea reducing habitats which support chemoautotrophic symbioses were discovered around hydrocarbon seeps in the Gulf of Mexico and at a cold seep off the Florida escarpment (Brooks et al., 1985; Hecker, 1985). The search for and discovery of other reducing habitats harboring chemoautotrophic symbioses continues to this day. Thus, the diversity and biological richness of sulfidic reducing habitats went from being unknown to a state of rapidly expanding knowledge within a very few years. The discovery of the vent communities created a paradigm shift in which sulfidic marine habitats were no longer looked at as harsh habitats requiring anaerobic adaptations for survival, but instead as resource-rich habitats suitable for a variety of taxa which possess chemoautotrophic symbioses.

From shortly after the discovery of the sulfur-oxidizing chemoautotrophic symbioses, there was also active pursuit of symbioses with chemoautotrophic bacteria using reductants other than sulfur compounds. This research was not successful until the discovery of methanotrophic symbionts in
hydrocarbon seep mussels (Childress et al., 1986), cold seep mussels (Cavanaugh et al., 1987), and a pogonophoran (Schmaljohann and Flügel, 1987). Even more recent studies have found symbioses in which both methanotrophic and sulfur-oxidizing symbionts are present simultaneously (Fisher et al., 1993; Schmaljohann et al., 1990). No reductants other than methane and sulfur compounds have been documented in these symbioses. Regardless of the reductant used or whether the animal is even a symbiotic one, however, all animals living in these reducing habitats have substantial exposure to sulfide.

The Study of Physiological Mechanisms for Handling Sulfide

By the time of the second hydrothermal vent biology expedition in 1982, in the middle of the age of discovery described above, it was clearly apparent that animals from vents and other reducing habitats were exposed to appreciable levels of sulfide. Indeed it was obvious that for those animals with sulfur-oxidizing symbionts, potentially toxic sulfur compounds had to pass through animal tissues to reach the symbionts. This was especially clear in the vestimentiferan tubeworm *Riftia pachyptila* whose symbionts were located in the animal’s trunk well removed from the gas exchange organ, the plume. These problems concerning sulfide led first to a consideration of how the symbioses dealt with sulfide and later to how the non-symbiotic species in these habitats avoided its toxicity. For the symbioses, the need to furnish sulfide compounds to the symbionts was an especially compelling problem which led to extensive studies. The dramatic and unexpected nature of many of the adaptations of the symbiotic animals to life with sulfide has served to increase the level of interest in all sulfide adaptations by making this a field which has many complex and fascinating stories (Somero et al., 1989). I will review some of the most dramatic of these findings below.

In the case of *Riftia pachyptila*, the circulating hemoglobin was found to bind sulfide reversibly, enabling the animal to concentrate sulfide from the water into the blood while maintaining the sulfide at a low activity and preventing spontaneous oxidation of sulfide by oxygen (Arp, 1986; Arp and Childress, 1983; Childress et al., 1984; Fisher and Childress, 1984). This sulfide-binding capability also served to protect the animal tissues and the symbionts from sulfide toxicity (Fisher et al., 1988; Powell and Somero, 1983). Later studies indicated that the endosymbionts of this animal could only use sulfide and not oxidation products such as thiosulfate (Fisher et al., 1989; Wilmot and Vetter, 1990) and that there were only very low concentrations of sulfide oxidation products in the blood of this species (Childress et al., 1991). Thus, vestimentiferan tubeworms appear to be specialized for the transport of sulfide to their symbionts, maximizing the reducing power delivered to the symbionts (Childress and Fisher, 1992). Their adaptations to life with sulfide appear to be the extreme case of accommodation and utilization of sulfide (Childress and Fisher, 1992).

At the same time, investigations of other vent and non-vent symbioses showed that a more common pattern was for the host to oxidize some or all of the sulfide which entered its body to thiosulfate and to supply that to the symbionts. In the case of the hydrothermal vent mussel, *Bathymodiolus thermophilus*, this may be the only sulfur compound used by the symbionts (Belkin et al., 1986). In vesicomyid clams, both from vents and other habitats, part of the sulfide taken up appears to be transported as sulfide bound to a zinc-containing serum component, and part is oxidized to thiosulfate, with the symbionts able to use both forms (Belkin et al., 1986). In vesicomyid clams, both from vents and other habitats, part of the sulfide taken up appears to be transported as sulfide bound to a zinc-containing serum component, and part is oxidized to thiosulfate, with the symbionts able to use both forms (Arp et al., 1984; Childress and Fisher, 1992; Childress et al., 1993; Childress et al., 1993). In other cases such as *Solemya reidi*, thiosulfate also seemed to predominate as the sulfur compound supplied to the symbionts (Anderson et al., 1987). In the case of *Solemya*, the spectacular finding was made that the animal mitochondria are capable of oxidizing sulfide with the production of ATP (O’Brien and Vetter, 1990; Powell and Somero, 1985; Powell and Somero, 1986). Thus, it is possible that in some cases the animals may receive benefits from sulfide.
oxidation outside those provided by bacterial symbionts.

The actual net autotrophic functioning of two symbioses while intact has been demonstrated using measurements of the fluxes of sulfide, oxygen, and carbon dioxide under various conditions. For Solemya reidi and Riftia pachyptila sulfide clearly drives net uptake of inorganic carbon, and oxygen is required for the process (Anderson et al., 1987; Childress et al., 1991). The rates of uptake can be quite high reaching 0.24% of body carbon per day for Solemya and 1.4% per day for Riftia. This and other observations led to the suggestion that Riftia growth should be extremely rapid at vents and lead to early domination of vent sites on the East Pacific Rise (Childress, 1988; Childress et al., 1991; Fustec et al., 1988).

More recent studies on the physiology of vent symbioses have focused on the physiological properties of the symbionts, the possible importance of alternative oxidants such as nitrate, the uptake of carbon and nitrogen by the hosts, and the role of ion exchange mechanisms at the host respiratory surfaces.

For non-symbiotic vent species, the questions relating to sulfide are much simpler. The primary interests have been in how the animal protects itself against the toxic effects of sulfide, how it gets rid of the products of detoxification, how it obtains oxygen, and whether it can make ATP from the oxidation of sulfide. The vent crab, Bythograea thermydron, has a remarkable resistance to sulfide, being able to tolerate at least 1.4 mM $\Sigma H_2S$ with little effect (Vetter et al., 1987). This tolerance is apparently achieved by oxidizing the sulfide to thiosulfate and eliminating it across the gills (Gorodezky and Childress, 1994; Vetter et al., 1987). The alvinellid polychaetes whose tubes encrust the walls of high temperature vents in some locations probably have the highest sulfide exposure, and are also exposed to the highest temperatures (Chevaldonné et al., 1992; Desbruyères et al., 1983; Gaill, 1988). These worms have substantial bacterial growths on the surfaces of their bodies, and interact with the vent fluids to form their tubes or to precipitate toxic materials (Juniper et al., 1986). As with the symbiotic species, by their example of surviving in the extreme vent environments, these organisms have inspired work on more accessible species exposed to sulfide.

With the stimulus of the studies on the spectacular hydrothermal vent animals a great deal of work on animals in more accessible sulfidic marine environments has been underway since early in the history of vent research. This work has made many unique and fascinating findings concerning adaptations of animals to life with sulfide. For example, studies on gill hemoglobins in lucinid and solemyid bivalves have indicated that there is an important role for these hemoglobins in regulating the intracellular environment of these clams (Doeller et al., 1988; Kraus and Wittenberg, 1990; Kraus et al., 1990; Wittenberg, 1985). With regard to sulfide, these hemoglobins react in a variety of previously undescribed ways suggesting that they may have a role in the supply of sulfide to the symbionts. Some non-vent non-symbiotic animals from sulfidic environments also appear to be excellent material for the study of the potential for animals to produce ATP based on sulfide oxidation, an exciting possibility (Kraus et al., 1991).

An area of especially great activity has been the study of interstitial animals. New discoveries of chemoautotrophic symbioses with annelids and nematodes have been made in this habitat (Giere, 1985; Giere et al., 1984; Polz et al., 1992). In addition to the discovery of these symbioses, much work has been carried out on the functioning of the annelid symbiosis describing its anatomy, its dependence on thiosulfate, and its impact on the ecology of the worms (Giere, 1989; Giere et al., 1991; Giere et al., 1988). There has also been active research on the non-symbiotic meiofauna including studies of sulfide toxicity, oxygen detoxifying enzymes, and the effects of sulfide on adenylate pools (Fox and Powell, 1986; Morrill et al., 1988; Powell et al., 1980). Especially significant is the hypothesis that thiobiotic meiofauna are sulfide-insensitive first-order respirers (Powell, 1989).

There has also been active research on animals burrowing in mudflats. This work includes studies of toxicity, sulfide perme-
ability, and sulfide effects on metabolism of a variety of animals. For example, in the echiuran worm, *Urechis caupo*, the epithelium has been shown to be readily permeable to H$_2$S necessitating defenses against sulfide (Julian and Arp, 1992). Hematin in this species' coelomocytes has been implicated in the oxidation of sulfide for detoxification and concentrations of hematin in coelom correlated with environmental sulfide concentrations (Arp et al., 1992; Eaton and Arp, 1993; Powell and Arp, 1989).

Although the major directions of current research have been identified above, this literature is rapidly expanding as interest in sulfide effects takes hold, now that the importance of sulfide has been brought to the attention of the community by the dramatic nature of the hydrothermal habitats and fauna. Further, the research in many different habitats has shown that adaptations to sulfide are widespread and far from being well understood at this time.

**Conclusions**

Because of the pervasive presence of sulfide and its high toxicity, virtually all organisms have some exposure to it and have some adaptations to deal with it. These mechanisms are much more highly developed in benthic marine environments where biologically or geologically produced sulfide is common. In the most extreme marine reducing environments the symbioses turn this problem into a resource which supports high rates of growth indeed. The discovery of the vent communities stimulated research on life with sulfide for marine animals in general because they demonstrated that not only was sulfide exposure a challenge to the survival of organisms, but that it could also be an opportunity to exploit a rich resource.

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