



Quaternary Ammonium Compounds as Candidate Photoprotective Compounds in Reef-Building Corals

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Quaternary ammonium compounds (QACs) – e.g., betaines – have a chemical structure related to that of the tertiary sulfonium compounds (TSCs) – e.g., dimethylsulfoniopropionate – explaining why these two classes of coral metabolites are often studied and interpreted together. Functionally, both QACs and TSCs play important roles in the photobiology of reef-building corals under stress, according to recent hypotheses. The TSC dimethylsulfoniopropionate (DMSP) is the principal precursor of the gas dimethylsulfide (DMS) which is hypothesized to affect, through influences on cloud formation, the photon and thermal fluxes to which corals are exposed. Simultaneously, QACs – e.g., glycine betaine – in coral tissues are hypothesized to protect the zooxanthellae photosystems against photon and thermal stresses by exerting stabilizing effects on photosystem proteins and by ameliorating reactive-oxygen-species perturbations. This review, which synthesizes the most current available evidence on the relevant actions of QACs, emphasizes the need for enhanced direct study of QAC physiology in corals to ascertain the degree to which coral QACs exert photoprotective effects paralleling their well-established protective effects in plants.

Keywords: abiotic stress, betaines, bleaching, glycine betaine, osmolytes, photon stress, photosynthesis, Tridacnidae

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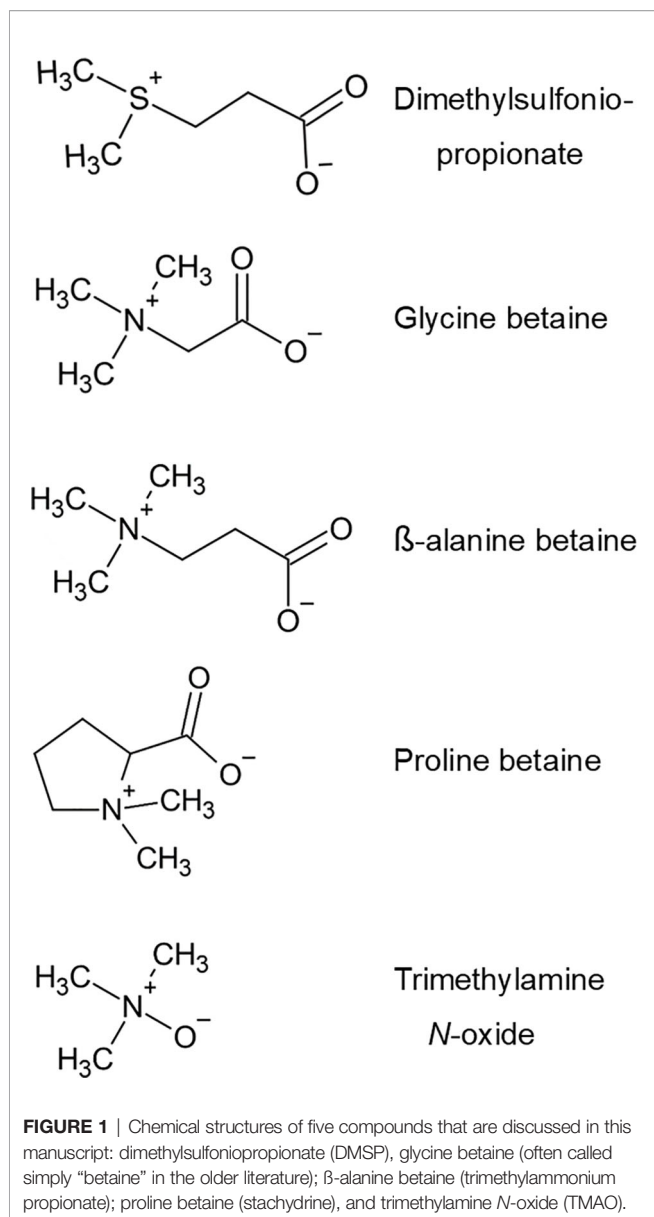
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INTRODUCTION

In a watershed paper, Rhodes and Hanson (1993) emphasized the logic and value of considering the tertiary sulfonium compounds (TSCs), notably dimethylsulfoniopropionate (DMSP), and the quaternary ammonium compounds (QACs) together because of their similarities in chemical structure and properties. Twenty-five years later, with far more data available, Somero et al. (2017) again affirmed the logic of emphasizing the shared properties of these two sets of small molecules, which they termed the methylsulfonium and methylammonium compounds – terms highlighting the key positions the compounds often occupy in methyl trafficking in cells. In this Special Issue on dimethylsulfide (DMS) in coral reefs, this paper on QACs is included not only because of the shared chemical properties of TSCs and QACs (**Figure 1**), but also because of important ways in which the two sets of compounds may interact in the ecological physiology of reef-building corals.

Glycine betaine is probably the most thoroughly studied QAC. Mäkelä et al. (2019) document that published research on glycine betaine has more than doubled in each successive decade over the past five decades, with more than 25,000 papers published in 2011–2020. Simultaneously, fewer than



20 papers have been published on glycine betaine or other QACs in reef-building corals over the entire five decades. In this paper, I argue that the near-total neglect of glycine betaine and other QACs in research on corals may represent a serious omission in the effort to conserve coral-reef ecosystems.

The betaines are one of the principal subsets of QACs. Structurally speaking, betaines are amino (or imino) acids fully methylated at the N position; there are in principle as many betaines as there are amino acids. Until a few decades ago, the most common betaine, glycine betaine, was typically called simply “betaine”. However, in this paper – and usually in the current scientific literature – it is called “glycine betaine” to specify the molecule and distinguish it from other betaines (although it is still called “betaine” in nontechnical contexts, such as ingredient lists for consumer products). In addition to glycine betaine, a number of other betaines, such as proline betaine and β -alanine betaine,

are commonly detected in vascular plants and algae when looked for (Rhodes and Hanson, 1993; McNeil et al., 1999). Trimethylamine N-oxide (TMAO) is a chemically related compound, long known in animals but only recently identified in plants (Catalá et al., 2021). All these compounds are zwitterionic, with a permanent positive charge at the N position: a structure that, as emphasized by Rhodes and Hanson (1993), is analogous to that of the TSC dimethylsulfoniopropionate, which is fully methylated at the S position and bears a permanent positive charge at that position (**Figure 1**).

A principal reason for glycine betaine to be of interest to coral biologists is its potential importance for photosynthesis. Already in 1995, Papageorgiou and Murata (1995) stressed the “unusually strong stabilizing effects of glycine betaine” on photosystem II in vascular plants. Other papers – such as those by Sakamoto and Murata (2002) and Allakhverdiev et al. (2003) – soon reinforced that message, helping to thrust glycine betaine into the center of research on photosynthesis under abiotic stress in vascular plants.

For discussing betaines and other QACs today, it is instructive to recognize the ways in which knowledge of the roles played by these compounds in photosynthetic organisms has expanded over the last several decades. In the 1970s and 1980s, glycine betaine was recognized principally as a compatible solute: an osmolyte that tended not to disturb cellular function (Yancey et al., 1982). As years passed, investigators identified additional roles, including glycine betaine’s stabilization of proteins in photosynthesis already alluded to (and addressed in detail later) and glycine betaine’s favorable effects (Chen and Murata, 2011) on reactive oxygen species (ROS) (Lesser, 2006). In the last few years, investigators have increasingly emphasized evidence that glycine betaine in addition can play signaling roles, as by directly or indirectly controlling gene expression (Figueroa-Soto and Valenzuela-Soto, 2018; Dutta et al., 2019; Hossain et al., 2019; Mäkelä et al., 2019; Valenzuela-Soto and Figueroa-Soto, 2019), and glycine betaine can modulate heat-shock protein expression (Li et al., 2011; Zhang et al., 2020). Today, therefore, when we consider glycine betaine and other QACs, we recognize that they may have broad ranges of action and be multifunctional (Derakhshani et al., 2017).

BETAINES IN REEF-BUILDING CORALS

Only about a decade has passed since publication of the first definitive information on betaines in the tissues of reef-building corals. The very first information seems to have been a report in 1976 by Moore and Huxley (1976) that crown-of-thorns seastars (*Acanthaster planci*) exhibit aversive behavior when exposed to a compound, tentatively identified as glycine betaine, in the tissues of certain reef-building corals. In 2010, Hill et al. (2010) and Yancey et al. (2010) used rigorous chemical methods [liquid chromatography/mass spectrometry (LC/MS) with internal standards in the case of Hill et al.] to quantify multiple betaines in the tissues of wild-collected, reef-building corals in the Caribbean (Hill et al., 2010) and at Hawaii (Yancey et al., 2010). Later, Hill et al. (2017), working mostly with wild-collected specimens, quantified multiple betaines in coral

species in the western Pacific and also in five species of tridacnid clams (genera *Tridacna* and *Hippopus*), animals that inhabit Indo-Pacific coral-reef ecosystems and, like corals, live in symbioses with dinoflagellate symbionts. Swan et al. (2017a) also quantified multiple betaines in a set of wild-collected Great Barrier Reef branching corals.

The evidence shows that betaines, despite their relative obscurity in corals, are abundant metabolites in the tissues of reef-building corals in the wild. Among the 10 species of Caribbean corals (6 genera) and the 6 species of western Pacific corals (5 genera), collected from the wild, that Hill et al. (2010; 2017) analyzed using LC/MS with internal standards, total betaine concentration was estimated to be 12–204 mmole per liter of living tissue (mean: 60 mM), except in one species, *Acropora formosa*, in which the total concentration was much lower (1.5 mM). An important point to stress is that these concentrations were calculated assuming a homogeneous distribution of the betaines in tissue. The true, operative concentrations of betaines in living tissue are likely substantially higher because betaines are probably, in fact, more concentrated in some subcellular regions than others. This same consideration applies also to other values reported.

Yancey et al. (2010), using a somewhat less sensitive analytical method than Hill et al. (2010; 2017), measured two betaines, glycine betaine and proline betaine, in 6 wild-collected species (4 genera) of Hawaiian reef-building corals and found total concentrations of about 9–69 mM (mean: 37 mM). Swan et al. (2017a), using a high-sensitivity method, focused on a restricted part of the anatomy, the branch tips, in 6 species of *Acropora* and one of *Stylophora*. Most of their estimates of total betaine concentration were 12–52 mM, although in *A. valida*, their estimate was lower, 6 mM.

Considering all studied coral species as a set, the two most abundant betaines in reef-building corals are glycine betaine and proline betaine. In the three studies that attempted to measure all betaines present in wild-collected corals (Hill et al., 2010; Hill et al., 2017; Swan et al., 2017a), glycine betaine and proline betaine together accounted for $\geq 90\%$ of all betaines (on a molar basis) in 14 species, 58–88% in 8 species, and $< 50\%$ in 1 species. Multiple additional betaines (e.g., alanine betaine, β -alanine betaine, and hydroxyproline betaine) are also typically present in reef-building corals, some at just low concentrations. Different coral taxa sometimes differ substantially in the particular suite of betaines they express.

Ngugi et al. (2020) recently completed a genomic survey of pathways for glycine betaine synthesis, transport, and degradation in a wide sample of invertebrates, including reef-building corals. According to the genomic evidence, corals have pathways for the *de novo* synthesis of glycine betaine and for transport of glycine betaine into their tissues from the seawater environment. The transporters in particular have a restricted phylogenetic distribution within the invertebrates, suggesting a specialized role in corals and/or coral relatives. Strikingly, Ngugi et al. (2020) calculated that corals worldwide store sufficient glycine betaine to account for 16% of their tissue nitrogen.

POTENTIAL PROTECTIVE EFFECTS OF BETAINES IN REEF-BUILDING CORALS: THE MESSAGE FROM PHYSIOLOGICAL DATA ON VASCULAR PLANTS AND ECOLOGICAL DATA ON CORALS

In corals, the biochemical and physiological actions of betaines have yet to be directly studied. However, the situation in vascular plants is quite opposite: By virtue of the massive effort to understand photosynthesis under abiotic stress in crop plants, a great deal is known about protective actions of glycine betaine. By applying the knowledge from vascular plants to reef-building corals, it seems highly reasonable to hypothesize that glycine betaine and other betaines are important photoprotective, protein- and membrane-stabilizing, compounds in corals. Should this hypothesis prove correct, manipulations of betaines (or other QACs) might be useful interventions for protecting reefs against threats of photoinhibition and bleaching (Gorbunov et al., 2001; Lesser, 2011; Frieler et al., 2013; Van Oppen and Lough, 2018), or for aiding recovery. Clearly, the tissue betaine concentrations measured in corals (reviewed in the previous section) are of sufficient magnitude for stabilization of protein and membrane functions, judging by studies in plants (e.g., Prasad and Saradhi, 2004; Shirasawa et al., 2006; Yang et al., 2007; Chen and Murata, 2011).

Before going further to explore these ideas, a few comments are appropriate regarding the fact that, at this time, although most research on the functions of betaines in photosynthetic organisms has been carried on one chemical species (glycine betaine), in the literature betaines are often discussed as a group: as a set of chemical species that, to a first approximation, have similar actions in protecting proteins and membranes. At the present time, there are two reasons for considering betaines as a group with common properties. First, several betaines in addition to glycine betaine, including β -alanine betaine and proline betaine (**Figure 1**), have been demonstrated in at least limited ways to act as compatible solutes (Rhodes and Hanson, 1993; Bashir et al., 2014). Second, existing theories of betaine action emphasize molecular properties that are shared by betaines as a group rather than being highly specific to certain chemical species. Specifically, the protein- and membrane-stabilizing effects of betaines are attributed for the most part to influences that betaines noncovalently exert – by virtue of their fundamental chemical nature – on the structure of water in the immediate vicinity of protein molecules, enhancing thereby the extent to which native protein states are more favorable thermodynamically than nonnative states (McNeil et al., 1999; Bennion and Daggett, 2004; Street et al., 2006; Auton et al., 2011; Guinn et al., 2011; Bruździak et al., 2013; Roychoudhury et al., 2013). At the present state of knowledge, it seems parsimonious and reasonable to hypothesize that (1) all the principal betaines in corals exert, to a significant extent, similar protective actions in corals and (2) as a corollary, the total betaine concentration in tissues is likely a useful index of betaine functional significance. Of course, looking forward, a full functional understanding of

betaines in corals will ultimately require direct empirical study of each specific chemical species.

For discussing betaines as potential protective compounds for photosynthesis in reef-building corals, two interacting lines of investigation on photosynthesis in vascular plants deserve particular attention: first, studies of photosystem II (PSII) and, second, studies of reactive oxygen species (ROS). Regarding PSII, abundant evidence exists that when corals are exposed to heat and photon stress sufficient to cause bleaching or photoinhibition, damaging effects are directly or indirectly exerted on PSII and/or the pathways of electron flow downstream from PSII in the algal symbionts of the corals (Warner et al., 1999; Fitt et al., 2001; Gorbunov et al., 2001; Jones and Hoegh-Guldberg, 2001; Hill et al., 2004; Lesser and Farrell, 2004; Weis, 2008; Warner and Suggett, 2016). Possibly the D1 protein (PsbA) in the PSII reaction center in coral symbionts – sometimes termed the Achilles heel of PSII (Weis, 2008) – is particularly affected (Warner et al., 1999; Hill et al., 2011; Warner and Suggett, 2016). As already emphasized, potential protective actions of glycine betaine or other QACs have not yet been directly studied in corals. However, in vascular plants and free-living algae that have been studied, glycine betaine has been demonstrated by many studies to protect PSII against a number of abiotic stresses, including photon stress and heat stress (Papageorgiou and Murata, 1995; Yang et al., 1996; Schiller and Dau, 2000; Sakamoto and Murata, 2002; Allakhverdiev et al., 2003; Klimov et al., 2003; Prasad and Saradhi, 2004; Hema et al., 2007; Yang et al., 2007; Allakhverdiev et al., 2008; Chen and Murata, 2011; Li et al., 2014; Wang et al., 2014; Kurepin et al., 2015; Huang et al., 2020; Li et al., 2021).

Regarding ROS, when corals are exposed to heat and photon stress sufficient to cause bleaching or photoinhibition, abundant evidence points to accumulation in the coral tissues of ROS, which are generally judged to be principal agents of photodamage (Lesser, 2006; Weis, 2008; Lesser, 2011; Oakley and Davy, 2018). Again, potential protective actions of glycine betaine or other QACs have not yet been directly studied in corals. Glycine betaine, however, has repeatedly been demonstrated to increase photosystem defenses against ROS in vascular plants (Prasad and Saradhi, 2004; Yang et al., 2007; Chen and Murata, 2011; Fan et al., 2012; Li et al., 2014; Gómez et al., 2019; Zhang et al., 2020; Li et al., 2021).

The research on glycine betaine in vascular plants has often had a very practical goal: to enhance the productivity of crop plants by capitalizing on glycine betaine's beneficial properties. As part of this effort, glycine betaine has long occupied a focal position in the overall effort to employ genetic modification, or other deliberate manipulation, to offset the negative impacts on crop plants of a variety of abiotic stresses, including photon stress and heat stress (Alia et al., 1998; McNeil et al., 1999; Sakamoto and Murata, 2002; Prasad and Saradhi, 2004; Yang et al., 2007; Chen and Murata, 2011; Giri, 2011; Li et al., 2011; Li et al., 2014; Kurepin et al., 2015; Castiglioni et al., 2018; Annunziata et al., 2019; Dutta et al., 2019; Hasanuzzaman et al., 2019; Kido et al., 2019; Zhang et al., 2020; Niazian et al., 2021). Recent review

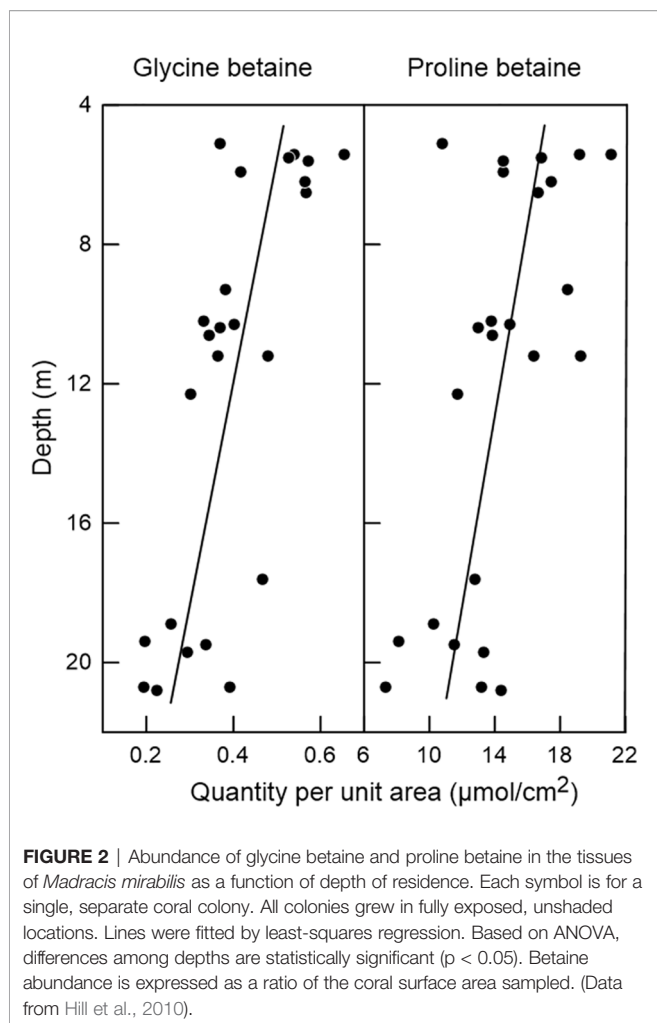
papers provide overviews on the biology and manipulation of glycine betaine from this perspective (Kurepin et al., 2015; Annunziata et al., 2019; Hasanuzzaman et al., 2019; Huang et al., 2020; Zulfiqar et al., 2020).

Ecological studies on coral reefs bolster the hypothesis that glycine betaine and other betaines are important photoprotective compounds in reef-building corals. The ecological studies represent a second major line of investigation pointing in this direction, in addition to the extrapolations from physiological studies of vascular plants already discussed.

Hill et al. (2010) hypothesized that within a population of a species of reef-building corals subject to potential photon stress, if tissue betaines are in fact protective agents for algal symbiont function [by providing either direct protection to the algal cells or indirect protection (e.g., stabilization of the symbiosome membrane)], betaine concentrations would be expected to vary among individual coral colonies in direct relation to solar irradiance experienced. For testing this hypothesis, Hill et al. (2010) selected *Madracis* species in Curaçao reefs because the light relations of those species had previously been studied in exceptional detail (Vermeij and Bak, 2002). *M. mirabilis* occurs over a wide range of depths and almost always occupies fully exposed (unshaded) locations regardless of depth (Vermeij and Bak, 2002). With this information, Hill et al. (2010) hypothesized *a priori* that betaine concentrations in colonies of *M. mirabilis* vary inversely with depth of residence. In fact, when measured, the concentrations of glycine betaine and proline betaine (**Figure 2**) – and also alanine betaine and hydroxyproline betaine – were found to vary significantly with depth, being 37–94% higher in coral colonies at 5 m depth than ones at 20 m (Hill et al., 2010). Glycine betaine and proline betaine are the most abundant betaines in *M. mirabilis*. Another *Madracis* species, *M. pharensis*, occupies both fully exposed and highly shaded locations at most depths where it occurs (Vermeij and Bak, 2002). Hill et al. (2010) hypothesized *a priori* that, at any particular depth, betaine concentrations are higher in exposed colonies than in shaded ones. In fact, when *M. pharensis* colonies at a single depth (10 m) were studied (Hill et al., 2010), glycine betaine, proline betaine, and alanine betaine were found to be significantly higher in concentration (by 30–44%) in exposed colonies than in shaded ones. Glycine betaine and proline betaine are by far the most abundant betaines in *M. pharensis*.

Reinforcing the ecological evidence for a protective role for glycine betaine in *Madracis*, Swan et al. (2017a) studied osmolyte concentrations in the branch tips of three *Acropora* species (*A. aspera*, *A. millepora*, and *A. valida*) during summer (February) and winter (August) in the Great Barrier Reef. They observed a 3.7- to 7.8-fold increase in average glycine betaine concentration in summer, compared with winter, in all three species: the pattern predicted if glycine betaine is employed in photoprotection. Although proline betaine was also abundant in these species, it exhibited just relatively small, inconsistent differences in concentration between the summer and winter samplings.

In summary, existing physiological and ecological evidence justifies a hypothesis that betaines play interacting



photoprotective and ROS-protective roles in reef-building corals. In my view, recognizing the dire threats now faced by corals (Frieler et al., 2013), it would be logical to place a high priority on carrying out direct tests of this hypothesis in the near future (Hill et al., 2010; Hill et al., 2017). Lesser (2011) and others have emphasized that throughout the history of studies on photosynthesis in reef-building corals, investigators have made great progress by using prior studies on vascular plants and free-living algae as guides. Today, based on a large literature (Mäkelä et al., 2019), glycine betaine is well known in the study of vascular plants as a metabolite that exerts multiple effects – notably protein-stabilizing, membrane-stabilizing, and ROS-ameliorating effects – that help defend plants stressed by high irradiance, unusual temperatures, and other agents. Because this knowledge has been sought principally with the practical objective of defending crop plants against abiotic stress, many published attempts have been made – often with successful results – to manipulate glycine betaine to advantage, either by exogenous application or by genetic manipulation of endogenous synthesis (Alia et al., 1998; McNeil et al., 1999; Sakamoto and Murata, 2002; Prasad and Saradhi, 2004; Yang et al., 2007; Chen and Murata, 2011; Giri, 2011; Li et al., 2011; Li et al., 2014;

Kurepin et al., 2015; Castiglioni et al., 2018; Annunziata et al., 2019; Dutta et al., 2019; Hasanuzzaman et al., 2019; Hossain et al., 2019; Huang et al., 2020; Zhang et al., 2020; Niazian et al., 2021). Perhaps this background of experience will prove useful for the protection of coral reefs confronted with photosystem and ROS stress (Van Oppen and Lough, 2018).

Just recently, trimethylamine *N*-oxide (TMAO; **Figure 1**) has been discovered to be widespread in vascular plants, and evidence from *Arabidopsis* and tomato indicates that TMAO, like betaines, plays roles in protein stabilization and tolerance of abiotic stress (Catalá et al., 2021). If TMAO proves to be present in reef-building corals, it also should be investigated along with betaines for actions that have potential to aid coral survival.

PHYSIOLOGICAL AND ECOLOGICAL INTERACTIONS BETWEEN THE TSC SYSTEM AND THE QAC SYSTEM

If we assume for the moment that glycine betaine and other QACs exert protective effects in reef-building corals – paralleling their demonstrated protective effects in vascular plants – important potential interactions between QACs and TSCs in corals become apparent. These interactions could be manifest at an ecosystem level and/or within the tissues of particular coral polyps.

At an ecosystem level, long-term monitoring has revealed that the atmospheric concentration of DMS varies significantly in coral-reef ecosystems on hourly, daily, and seasonal time scales; and as the atmospheric DMS concentration varies, the atmospheric concentration of aerosols and the solar irradiance at sea level sometimes vary in correlated ways (Jackson et al., 2020a). At times when the atmospheric DMS concentration falls and the irradiance at sea level accordingly increases, corals could offset the increased risk of photosystem stress by upregulating glycine betaine or other QACs in their tissues. To assess the likelihood of this type of response, more research will be required on the time scales on which corals are able to modulate betaine expression.

At the level of an individual coral polyp, tissue QACs and TSCs seem likely to interact in ameliorating ROS damage, including ROS-mediated damage to photosystem components such as PSII. As earlier noted, tissue glycine betaine has repeatedly been demonstrated to enhance defenses against ROS in vascular plants and free-living algae (Prasad and Saradhi, 2004; Hema et al., 2007; Yang et al., 2007; Chen and Murata, 2011; Fan et al., 2012; Gómez et al., 2019; Li et al., 2021). Similarly, DMSP and DMS are recognized as ROS scavengers in corals (Sunda et al., 2002; Lesser, 2006; Deschaseaux et al., 2014; Jones and King, 2015; Oakley and Davy, 2018; Jackson et al., 2020b). Thus both tissue QACs and TSCs likely function in the amelioration of ROS damage in corals, and they may be coordinated in various ways. To illustrate, corals have been observed to outgas DMS during air exposure or other stresses of low tide, when exposed or nearly exposed corals are subject to relatively high solar irradiance (Swan et al., 2017b). The corals might upregulate tissue betaines to compensate for any reduction in tissue DMS.

These briefly described hypothetical scenarios help to articulate the potential ways in which the QAC and TSC systems interact in the lives of reef corals and other photosynthetic reef inhabitants such as the tridacnid clams (Hill et al., 2017).

CONCLUSION

In the study of photosynthesis, of all the focal topics receiving frequent attention in vascular plants, the actions of betaines and other QACs are among the most neglected in reef-building corals. This neglect in corals is regrettable because corals are existentially threatened by abiotic stresses, and betaines attract interest in the study of crop plants precisely because of evidence that they can reduce the impact of abiotic stresses.

Recent research demonstrates that betaines (particularly glycine betaine and proline betaine) are abundant metabolites in the tissues of reef-building corals. Their functions in corals have not yet been directly studied. However, two lines of investigation – physiological and ecological – point to a hypothesis that betaines are photoprotective in corals. The physiological evidence focuses on the fact that when corals are subjected to heat and photon stresses sufficient to cause bleaching or photoinhibition, they experience damage to photosystems (e.g., PSII) and they accumulate photodamaging ROS: exigencies known to be ameliorated by glycine betaine in vascular plants. The ecological evidence is that when free-living corals have been examined to detect correlations between betaine abundance and solar irradiance, glycine betaine and (in some cases) other betaines have been observed to be significantly more concentrated in the tissues of coral colonies exposed to high irradiance than in conspecific colonies exposed to lower irradiance.

Betaines are not simple to study, in part because of the complexity of technologies available for quantification (e.g.,

LC/MS) and the challenges of calibration (Hill et al., 2010; Swan et al., 2017a). Nonetheless, a large body of evidence exists on their manipulation (e.g., exogenous application and genetic manipulation) in crop plants to defend against abiotic stresses, and with this background of experience, it is reasonable to hypothesize that they could be manipulated to aid the survival of corals exposed to heat and photon stresses.

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RH composed this paper in its entirety and prepared the figures.

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