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Inhibition of Coral and Algal Photosynthesis by Ca^{2+}-Antagonist Phenothiazine Drugs[1]

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ABSTRACT

The effects of various calcium ion antagonists and ion transport inhibitors on photosynthetic O_2 evolution of corals, isolated zooxanthellae, sea anemone tentacles, and *Chlorococcum oleofaciens* were measured. Only the phenothiazine drugs were effective at inhibiting photosynthesis. Trifluoperazine, a calcium ion antagonist drug, inhibited at low concentrations, with 10^{-4} molar and 8×10^{-6} molar completely abolishing photosynthesis in the intact corals and isolated zooxanthellae, respectively. Net photosynthetic O_2 evolution of *C. oleofaciens* was eliminated by concentrations of trifluoperazine as low as 2.8×10^{-5} molar.

Seawater contains approximately 10 mM Ca^{2+}, an ion required for deposition of $CaCO_3$ in coral skeletal formation. This calcification process is more rapid in hermatypic corals (corals which contain unicellular dinoflagellate symbionts called zooxanthellae) than in ahermatypic corals which do not contain zooxanthellae. The high concentration of Ca^{2+} coupled with its role in coral formation stimulated our interest in studying certain aspects of Ca^{2+} metabolism in the marine environment.

We investigated the effects of various inhibitors on photosynthesis of intact hermatypic corals, isolated zooxanthellae, and other photosynthetic organisms. In particular, we examined what effects TFP[2] and other phenothiazine drugs have on photosynthetic O_2 evolution. We chose these drugs since, in the presence of Ca^{2+}, these drugs bind calmodulin (1, 7, 11), a Ca^{2+}-dependent activator protein of several enzymes, including at least three plant enzymes (1, 2, 7, 9, 18). This study is the first to examine the effects of phenothiazine drugs on an intact marine symbiosis, on isolated algae from the symbiosis, and on algal photosynthesis.

MATERIALS AND METHODS

In July and August of 1981, fresh specimens of the corals *Pocillopora damicornis* and *Seriatopora hystrix* were collected at a depth of 5 m from Herald's Prong #2 Reef (151°32.75′E, 21°14.52′S) on the Great Barrier Reef just prior to use in the experiments. Zooxanthellae were isolated from *P. damicornis* using the Water Pik method of Johannes and Wiebe (10). Isolated zooxanthellae or pieces of intact coral were placed in seawater in a Clark-type O_2 electrode (Rank Brothers, Cambridge, England), and O_2 evolution and consumption were measured. To prevent the pieces of coral from disrupting the motion of the stir bar, they were placed on a wire screen mounted in the O_2 electrode chamber above the stir bar. Coral pieces used in the O_2 electrode were always broken off a larger coral head immediately prior to being used in any experiments. The rates of photosynthetic O_2 evolution were measured at saturating light intensities (2.0×10^{16} quanta cm^{-2} s^{-1}) of white light provided by a GE 500-w bulb in a Graflex slide projector and were recorded on a Beckman chart recorder. Small amounts (5-50 µl) of various inhibitors of different concentrations were added directly to the seawater in the O_2 electrode chamber through a small hole in the lucite plug which forms the top of the chamber. These inhibitors included: NAP-taurine, DIDS, SITS, TFP, EGTA, and A23187. Then the effects of these inhibitors on photosynthesis were measured.

The effect of TFP on the freshwater green unicellular alga, *Chlorococcum oleofaciens* (UTEX 105) was measured in University Park, PA. *C. oleofaciens* was grown on CS medium (15) at 20°C under continuous light of 8.0×10^{15} quanta cm^{-2} s^{-1} provided by cool-white fluorescent lights. The cultures were bubbled continuously with air during growth. Photosynthesis was measured in a manner similar to that for the isolated zooxanthellae.

The effects of several phenothiazine drugs on photosynthesis of a sea anemone were studied in Athens, GA. The sea anemone, *Condylactis gigantea*, was cultured in seawater by John Patton, Microbiology Department, University of Georgia. Tentacles were snipped from the anemone and placed in seawater in an O_2 electrode chamber. O_2 exchange was measured at 9.0×10^{15} quanta cm^{-2} s^{-1} at 20°C. The phenothiazine drugs were prepared fresh and added directly to the seawater medium. O_2 exchange was followed for 10 to 15 min after adding the drug. Generally, 5 to 10 min were required for O_2 exchange to reach a steady value after drug addition. We assume that a time-dependent penetration by the drugs into the symbiont is the cause of the time-dependent response of O_2 exchange.

RESULTS

In our initial work, we studied the effects of various inhibitors on intact coral photosynthesis. Even at concentrations as high as 5×10^{-4} M, NAP-taurine, DIDS, SITS, and A23187 had no effect on photosynthesis. EGTA did inhibit photosynthesis, but only by 25% at a concentration of 7.5×10^{-3} M. In contrast, TFP inhibited photosynthesis at low concentrations (Fig. 1). We thus directed our research efforts at examining the effects of TFP.

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[2] Abbreviations: TFP, trifluoperazine; NAP-taurine, *N*-(4-azido-2-nitrophenyl)-2-aminoethylsulfonate; DIDS, 4,4′-diisothiocyano-2,2′-stilbenedisulfonic acid; SITS, 4-acetamido-4′-isothiocyano-2,2′-stilbenedisulfonic acid; EGTA, ethyleneglycol-bis(β-aminoethyl ether)-*N*,*N*′-tetraacetic acid; FP, fluphenazine; CP, chlorpromazine; CPS, chlorpromazine sulfoxide; DCIP, 2,6-dichloroindophenol.

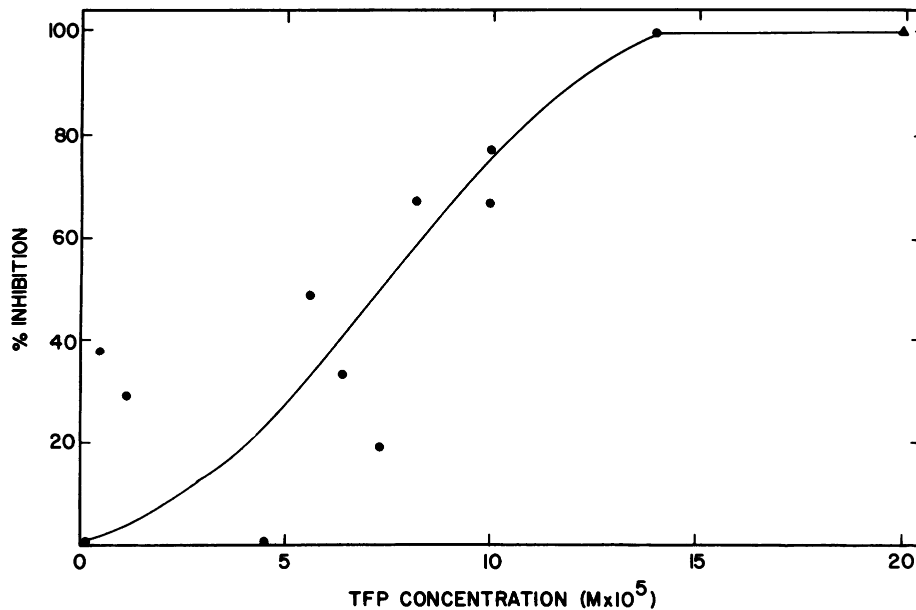

FIG. 1. Percent inhibition of intact *S. hystrix* photosynthetic O_2 evolution by various concentrations of TFP. O_2 uptake in the light of 5 µmol O_2/mg Chl *a*·h is represented by (▲). The results presented are from three different experimental runs using different pieces of coral. The control rates of photosynthesis ranged from 40 to 230 µmol O_2/mg Chl *a*·h.

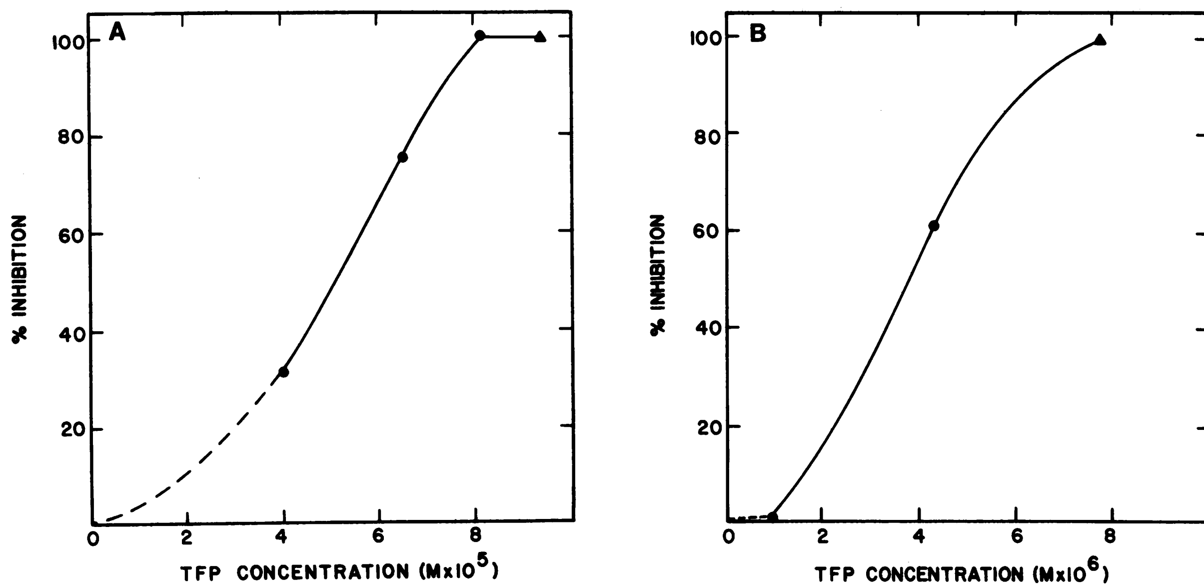

FIG. 2, A and B. Percent inhibition of intact *P. damicornis* photosynthetic O_2 evolution by various concentrations of TFP. O_2 uptake in the light of 95 µmol O_2/mg Chl *a*·h is represented by (▲). The control rate of photosynthesis was 300 µmol O_2/mg Chl *a*·h. B, Percent inhibition of photosynthetic O_2 evolution of zooxanthellae isolated from *P. damicornis* by various concentrations of TFP. O_2 uptake in the light of 11 µmol O_2/mg Chl *a*·h is represented by (▲). The control rate of photosynthesis was 108 µmol O_2/mg Chl *a*·h.

The effects of TFP on coral and zooxanthellae photosynthesis are summarized in Figures 1, 2A, and 2B. Quite low concentrations of TFP inhibited *Seriatopora hystrix* photosynthesis, with a concentration of 1.4×10^{-4} M completely preventing net O_2 evolution (Fig. 1). A concentration of 2.0×10^{-4} M further reduced total photosynthesis, with a net uptake of O_2 occurring in the light. A similar trend was seen both for intact *Pocillopora damicornis* arms (Fig. 2A) and the isolated zooxanthellae (Fig. 2B), which were about 10-fold more susceptible to inhibition than the intact coral. The photosynthesis of *C. oleofaciens* (Fig. 3) also was reduced in the presence of TFP, although the concentration required was somewhat higher than for the inhibition of isolated *P. damicornis* zooxanthellae (Fig. 2B).

TFP also inhibited the photosynthesis of *C. gigantea* tentacles (Table I). Other phenothiazine drugs, FP and CP, which have previously been shown to inhibit calmodulin activity (1, 9, 11), also inhibited photosynthesis. CPS, a phenothiazine derivative, had little consistent effect on photosynthesis (Table I). Previous work has shown that CPS does not affect calmodulin activity (11).

DISCUSSION

DIDS, SITS, and NAP-taurine have previously been shown to inhibit transport of the anions Cl^- and SO_4^{-2} in corn root protoplasts (12). These compounds also reduced the K^+-ATPase activity of the corn root plasmalemma (12). Since seawater contains 0.55 M Cl^- and 28 mM SO_4^{-2}, the inhibition of transport of these ions might be expected to have some effect on the photosynthetic

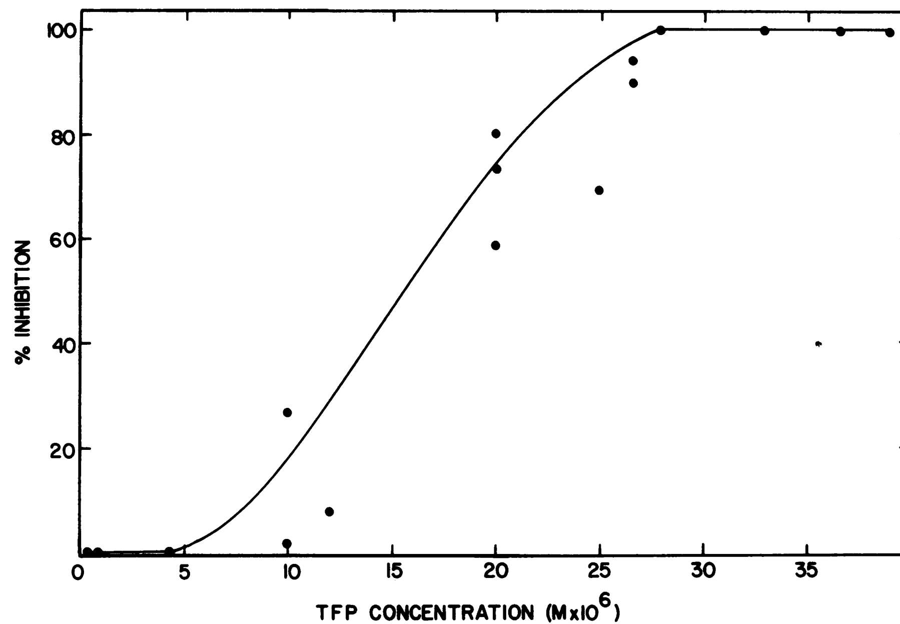

FIG. 3. Percent inhibition of *C. oleofaciens* photosynthetic O_2 evolution by various concentrations of TFP. The results presented are from six different experimental runs. The control rates of photosynthesis ranged from 65 to 380 μmol O_2/mg Chl *a*·h.

Table I. *Influence of Phenothiazine Drugs on Photosynthesis in Detached Tentacles of the Sea Anemone C. gigantea.*

Two sets of experiments are reported.

Drug	Concentration	O_2 Evolution
	M	*μmol/mg Chl·h*
Control		80, 53
+ TFP	10^{-4}	−8,[a] −21
Control		114, 69
+ FP	10^{-4}	−19, −12
Control		90, 79
+ CP	10^{-4}	−10, 15
Control		63, 129
+ CPS	10^{-4}	93, 86

[a] Negative values are O_2 uptake.

metabolism of marine organisms. This was not the case, as none of these inhibitory compounds affected photosynthesis, even at relatively high concentrations. The absence of an effect may reflect the lack of a carrier protein for Cl^- and SO_4^{-2} in the coral and zooxanthellae. These inhibitors also may not inhibit the carrier protein, if one exists, in the coral and zooxanthellae. Finally, the blocking of Cl and SO_4^{-2} transport in the coral and zooxanthellae may not affect photosynthesis, the only metabolic parameter we measured.

EGTA, a chelator of Ca^{2+}, might be expected to prevent Ca^{2+} interactions with various metabolic processes. At quite high concentrations (7.5 mM), an inhibition of photosynthesis was observed. The high concentration required for inhibition may indicate that an excess of Ca^{2+} for photosynthetic metabolism is present in seawater and only when much of the 10 mM Ca^{2+} in seawater is chelated does one begin to see an effect on photosynthesis.

A23187 is a Ca^{2+} and Mg^{2+} ionophore and therefore may facilitate the movement of Ca^{2+} into the coral and zooxanthellae cells. In our experiments, A23187 neither stimulated nor inhibited photosynthesis, perhaps indicating that Ca^{2+} was already present in sufficient quantities for photosynthesis.

Low concentrations of TFP, an antagonist of calmodulin, inhibited photosynthetic O_2 evolution in intact corals (Figs. 1 and 2A), in isolated zooxanthellae (Fig. 2B), in *C. oleofaciens* (Fig. 3), and in sea anemone tentacles (Table I). Higher concentrations of TFP (Figs. 1–2B; Table I) were sufficient to eliminate net O_2 evolution completely and to allow respiratory consumption of O_2 to be expressed. We assume that this O_2 consumption is caused by animal and zooxanthellae mitochondrial respiration which is always occurring, but which is usually not measured in the light because of the presence of photosynthetic O_2 evolution. We found no inhibition of intact *S. hystrix* mitochondrial respiration by 1.4 × 10^{-4} M TFP.

Our demonstration of this inhibition of photosynthesis represents the first published evidence that TFP can have such an effect on marine invertebrates and algae. This inhibition is similar to that observed by Barr *et al.* (5) who found an inhibition by TFP of PSII in spinach chloroplasts. They found a concentration of 100 μM TFP inhibited DCIP reduction by 75% and a concentration of 400 μM completely inhibited DCIP reduction. These inhibitory concentrations are similar to those we observed (Figs. 1–3).

The effect of TFP on photosynthesis may indicate an involvement of calmodulin in photosynthesis. TFP has been shown to be a calmodulin inhibitor (1, 7, 11) and thus, by implication, a calmodulin-like compound may be important in the photosynthetic process (5). Muto *et al.* (13) also have shown that a Ca^{2+}-calmodulin enzyme or some other Ca^{2+}-sensitive enzyme may be involved in the light activation of chloroplast enzymes, while Jarrett *et al.* (9) also have found a calmodulin-like protein in the stroma of pea chloroplasts.

Further evidence for the presence of a calmodulin-like compound important in photosynthesis is provided by our demonstration of the inhibition of sea anemone photosynthesis by FP and CP (Table I), two phenothiazine drugs also implicated as calmodulin inhibitors (9, 11), and by its relative insensitivity to CPS.

Caution must be exercised, though, in concluding that our results indicate the presence of calmodulin in zooxanthellae and *C. oleofaciens* or its importance in photosynthesis. TFP is often assumed to be a rather specific inhibitor of calmodulin, but as Cheung (7) has pointed out, TFP is a hydrophobic compound. Its hydrophobicity may lead to its interacting with other hydrophobic

compounds and affecting photosynthesis other than through calmodulin. As an example of such an effect, CP has been shown to uncouple photophosphorylation (3). Since TFP and FP are similar to CP, they also may affect the algal cells in this fashion. This type of an interaction probably would not be responsible for the decrease in O_2 evolution we observed, as photophosphorylation uncouplers generally cause an increase in photosynthetic O_2 evolution (16). A demonstration of calmodulin-regulated reactions requires more experiments that we have conducted. Cheung (7) has listed five criteria required to show that a reaction is calmodulin-regulated, only one of which is to study the effects of TFP.

We also may be studying a Ca^{2+}-regulated reaction which does not directly involve calmodulin. Barber (4) has reviewed many of the effects of ions and points out that Ca^{2+} may be a co-ion in H^+ transport into the thylakoids, may be involved in conformational changes in the thylakoids and in the uncoupling of electron flow, and may control the *in vivo* State 1-State 2 transitions.

Although our results are preliminary, we feel that the possible function of calmodulin in algal photosynthesis should be examined. For example, does an inhibition of calmodulin affect NAD kinase or other enzymes or is it more directly involved in photosynthesis?

In the marine environment, with its high Ca^{2+} concentration, the role of calmodulin in the calcification process is an intriguing question. Previous studies have shown light generally enhances calcification (6, 14) and that DCMU, an inhibitor of all O_2-evolving photosynthesis, inhibits light-enhanced coral calcifications (17). These observations coupled with the more rapid rates of coral skeletal growth in hermatypic *versus* ahermatypic corals indicate an important role of zooxanthellae and their photosynthesis in calcification. The exact mechanism of calcification remains uncertain though, in spite of a wide variety of proposed mechanisms (8, 14).

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