Oxygenic photosynthesis evolved in an anaerobic environment that contained several orders of magnitude more CO₂ than present-day air. As photosynthesis oxygenated the environment, the formation of reactive oxygen species (ROS) would have become more common. When oxygen is present, photosystem I can accidentally transfer a single electron to molecular oxygen, resulting in the formation of superoxide anions. Subsequently, superoxides can be converted to hydrogen peroxide and/or hydroxyl radicals. Additionally, excited singlet-state oxygen can form within both photosystems I and II (Asada and Takahashi, 1987). All ROS indiscriminately react with biological molecules, resulting in stress that can lead to cell death. Similar reactions occur with electron transport proteins (e.g., cytochromes) of non-photosynthetic organisms. The antioxidant system detoxifies ROS by adding additional electrons to radicals and quenching excited-state molecules (Fridovich, 1989). Therefore, the co-evolution of the antioxidant system and oxygenic photosynthesis presents a paradox. Without antioxidants, oxygenic photosynthesis is self-destructive. However, without photosynthesis, there may not have been any selective pressure for the evolution of antioxidants.

We considered two possible hypotheses regarding the sequence of photosynthesis-antioxidant co-evolution. If photosynthesis evolved first, the large diffusion gradient produced by the anoxic environment would have allowed oxygen to diffuse out of the cells before being converted to ROS. An antioxidant system would not be needed until the environment became oxygenated. Alternatively, antioxidant systems may have evolved first in response to UV-induced and other non-biogenic oxidative stresses. The presence of an antioxidant system would have protected earlier anoxygenic photosynthesizers, and provided a pre-adaptation for the later evolution of oxygenic photosynthesis.

To test these hypotheses, we grew wild-type and antioxidant-deficient strains of *Synechococcus* sp. PCC7942 in air and in simulated primordial atmospheres (2.5% CO₂ in N₂). The *katG* strain lacks catalase; the *tplA* strain lacks a thioredoxin-peroxidase-like enzyme (Perelman et al., 2003); and the *sodB* strain (Herbert et al., 1992) lacks iron superoxide dismutase (FeSOD). If the presence of intact antioxidant enzymes was required under primordial conditions, we would expect the growth rates of the wild-type strains to be higher than those of the antioxidant mutant strains. Conversely, if antioxidants were not needed under primordial conditions, we would expect the growth rates of the wild-type and mutant strains to be identical.

Cyanobacterial cultures were grown in liquid BG-11 medium at 27°C, and illuminated with cool-white fluorescent tubes at 50 µmol photons m⁻² s⁻¹. Chloramphenicol or spectinomycin was added as a selective agent to stock cultures of antioxidant deletion strains. Antibiotics were not added to experimental cultures. Control cultures were bubbled with filtered air; experimental cultures were bubbled with 2.5% CO₂ in N₂ (Thomas and Herbert, 2005). As a control for the effects of CO₂, additional cultures were bubbled with 2.5% CO₂ in air. Growth was measured as optical density at 720 nm. Damage to photosystem II was quantified by measuring chlorophyll fluorescence (Fv/Fm) (Campbell et al., 1998). Damage to photosystem I was measured by changes in P700 redox kinetics (Sacksteder and Kramer, 2000). Both photosystem measurements were made with a Walz PAM-101 chlorophyll fluorometer. Data were analyzed with Microsoft Excel 2003, α = 0.05.

Figure 1 shows the growth rates of the cyanobacteria. The *sodB* strain grew significantly slower than the other strains under all conditions, indicating that FeSOD provides a protective benefit even under anaerobic conditions.
After 72 hours of growth in air, the wild-type and katG strains had essentially identical Fv/FM values (not shown). However, the sodB strain had significantly elevated Fv/FM, indicating a healthier photosystem II apparatus. Conversely, the tplA strain had significantly lower Fv/FM, indicating greater inhibition of photosystem II. Cultures grown in 2.5% CO2/N2 showed no significant differences in Fv/FM. When cultures were grown in 2.5% CO2/air, only the sodB strain showed a significant increase in Fv/FM. The increased health of photosystem II seen in the sodB strain grown in the presence of O2 may be due to increased activity of the MnSOD (SodA), which has been reported in earlier research (Thomas et al., 1999). Overall, these results indicate that catalase (KatG) and thioredoxin peroxidase (TplA) provide no protective benefit to photosystem II under anaerobic conditions. TplA appears to provide some protection to photosystem II in air, but a small decrease in pO2 (2.5% CO2/air) appears to obviate the necessity of TplA.

When grown in air or 2.5% CO2/air, the wild-type and tplA strains had identical amounts of functional P700 (not shown). The katG strain had significantly more P700, while the sodB strain had significantly less P700. The sodB strain also had significantly less functional P700 under both CO2 atmospheres, indicating the FeSOD provided protection to photosystem I under both aerobic and anaerobic conditions. When grown under 2.5% CO2/N2, the katG and tplA strains had the same amount of functional P700 as the wild-type strain. The increase in functional P700 in the katG strain may be due to increased activity of TplA or some other antioxidant. Overall, the P700 data indicate that FeSOD provides protection to photosystem I under all conditions tested, while TplA is dispensable under anaerobic conditions.

From these results, we conclude that FeSOD protects photosystem I under both aerobic and anaerobic conditions, but cells grow (slower) without it. Catalase-peroxidase probably evolved before SODs since SODs produce H2O2. Thioredoxin peroxidase provides additional, but minimal, protection to photosystems I and II—the lack of TplA has no significant effect on growth in O2. Our results are confounded somewhat by the presence of the MnSOD (SodA) associated with the thylakoid membrane. A viable sodA mutant has not been reported in the literature, and attempts by DJT and S. K. Herbert (U. of WY) to produce such a mutant have been unsuccessful. A sodA deletion may be a lethal mutation under ambient conditions.

Based upon our findings and those of other researchers, we propose the following evolutionary sequence of events. Catalase evolved in response to exogenous, non-biologically produced H2O2 (Borda et al., 2001; McKay and Hartman, 1991). Duplication of the catalase gene allowed the evolution of a primitive anoxic photosystem from the catalase enzyme core while retaining catalase activity (Blankenship and Hartman, 1998). Depletion of reduced electron sources for anoxic photosynthesis provided selection pressure for the evolution of oxygenic photosynthesis. Internally-produced O2 and further oxygenation of the environment increased the production of O22, and selected for the evolution of SODs (this research). Increased internal production of H2O2 from SODs selected for additional peroxidases (thioredoxin peroxidase, ascorbate peroxidase, etc.) to supplement catalase-peroxidase (this research), and resulted in cells that resembled those of present-day cyanobacteria.

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REFERENCES


