CLIMATE CHANGE AND ITS INFLUENCE ON PHOTOSYNTHETIC BACTERIAL BLOOMS

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Summary

Environmental changes, air pollution and ozone depletion are increasing oxidative stress, and global warming threatens health by heat stress. We now face a high risk of simultaneous exposure to heat and oxidative stress. However, there have been few studies investigating their combined adverse effects on cell viability.

Singlet oxygen is one of several reactive oxygen species that can destroy biomolecules, microorganisms and other cells. Traditionally, the response to singlet oxygen has been termed photo-oxidative stress, as light-dependent processes in photosynthetic cells are major biological sources of singlet oxygen. Recent work identifying a core set of singlet oxygen stress response genes across various bacterial species highlights the importance of this response for survival by both photosynthetic and non-photosynthetic cells. Here, we review how bacterial cells mount a transcriptional response to photo-oxidative stress in the context of what is known about bacterial stress responses to other reactive oxygen species.

Keywords: Photo-oxidative stress; Reactive oxygen species (ROS); Carotenoid; Rhodobacter sphaeroides

Biochemical processes in photosynthesis:

The key enzyme in photosynthesis is Rubisco, ribulose bisphosphate carboxylase/oxygenase. There are four forms of this enzyme: form I found in all plants, most algae, cyanobacteria and some proteobacteria; form II found in other proteobacteria and dinoflagellate algae; form III in archaea; and form IV in some proteobacteria and archaea. Form IV Rubisco does not catalyse the classical photosynthesis reaction and is thus termed 'Rubisco-like protein'. It may be involved in methionine salvage pathways. The evolution of the various Rubisco proteins, concluding that the first Rubisco may well have evolved in amethanogen-like ancestor, acquiring new functions as it spread through lateral gene transfer [1]. For the Rubisco enzyme to fix carbon, it must have access to carbon dioxide. When oxygenic photosynthesis first evolved, presumably in small prokaryotes, simple diffusion into the cell provided a sufficient supply of carbon dioxide.

However, in a more productive biosphere with less carbon dioxide in the air, and as organisms became larger, simple diffusion was not able to supply sufficient carbon dioxide to the Rubisco proteins. The inorganic carbon concentrating mechanisms (CCMs) that catalyse the accumulation of CO_2 around Rubisco in all cyanobacteria, most algae and aquatic plants and in vascular plants with C_4 photosynthesis or Crassulacean acid metabolism [2]. The earliest CCMs may have evolved in oxygenic cyanobacteria in stromatolites before the atmosphere became oxygenated. In what setting did early photosynthesis evolve? The pre-photosynthetic niches were very meagre in productivity, far less than with modern photosynthesis [3]. Serpentinization, arc volcanism and ridge-axis volcanism reliably provided H_2, while early methanogens and acetogens reacted CO_2 with H_2 to obtain energy and make organic matter. A bacterium in such a setting may have been pre-adapted for anoxygenic photosynthesis, perhaps starting with H_2 in lieu of an oxygen 'acceptor'. After cyanobacterial oxygenic photosynthesis evolved, it appears to have been several million years before an oxygen-rich atmosphere developed. Eventually, anoxygenic and oxygenic photosynthesis oxidized much of the Earth's crust and supplied sulphate to the ocean. Once Rubisco has captured the carbon dioxide, an electron donor is obtained by splitting water, releasing oxygen into the atmosphere. Light-driven oxidation of water occurs at a catalytic centre contained within a multi-subunit enzyme known as photosystem II located in the photosynthetic membranes of plants, algae and cyanobacteria. Its oxygen-evolving centre (OEC) consists of four manganese ions and a calcium ion surrounded by a highly conserved protein environment. Barber (2008) shows how X-ray crystallography of photosystem II from the cyanobacterium Thermosynechococcus elongatus has revealed the structure to a resolution sufficient to determine the positions of almost all the amino acid residues and to show that the most probable structure of the OEC itself is a Mn_3CaO_4 cubane with the fourth Mn outside the cubane cluster. This, together with a wide range of information from biophysical and biochemical techniques, has made it possible to come very close to revealing the precise chemicalmechanism of the crucial water-splitting reaction of oxygenic photosynthesis. The close evolutionary relationship between the protein structure, and most of the cofactors, of photosystem II and all other photosystems of both oxygenic and anoxygenic organisms is apparent. But where did this unique $^4Mn/^1Ca$ complex come from? Its evolutionary origins, both inorganic and biological, remain an unsolved puzzle. Some of the associated proteins, such as PsbO, are also unique and seem to be restricted to the oxygenic photosystem. These proteins have clearly evolved for a sole purpose and have a unique beta-barrelled structure.

Photo-oxidative stress in bacteria:

Most studies on the microbial or cellular response to reactive oxygen species (ROS) have centred on protection from O_2 and H_2O_2. However, in the past decade our understanding of the response to $^1O_2$ (or to photo-oxidative stress) has greatly improved.

Evidence for photo-oxidative stress in bacteria:

Photo-oxidative stress and its consequences have been observed in numerous biological systems. The presence of photo-oxidative stress in bacteria was reported over 50 years ago, when the analysis of wild-type and carotenoid-deficient strains of the purple photosynthetic bacterium Rhodobacter sphaeroides showed that the presence of carotenoids protected the cells from so-called photodynamic damage that was caused by the introduction of air to photosynthetically growing cultures [7]. Decades later, the identity of the toxic byproduct of photodynamic damage was discovered when triplet excited bacteriochlorophyll a(3Bchla*) was identified as the light-

excited sensitizer that reacts with O_2 to produce $^1O_2$ [5,6,8]. Carotenoids, along with Bchla, are components of the photosynthetic apparatus, both in photosynthetic reaction centres (the bacterial ancestors of photosystems in O_2-evolving phototrophs) and light-harvesting complexes [6]. Carotenoids have been shown to directly quench $^1O_2$, but their primary protective effect in vivo is likely to be the rapid quenching of 3Bchla* to reduce $^1O_2$ formation [6,8].

Evidence for the cellular adaptation to photooxidative stress

The cultures of strain TC67 and wild-type cultures recovered from low growth rates during prolonged incubation under low light and photo-oxidative stress, respectively. This suggests that an adaptation to the stress conditions has occurred. However, the spontaneous generation of mutants more resistant to photo-oxidative stress may also explain this phenomenon.

In a previous study with carotenoid-deficient mutants of Rubrivivax gelatinosus, a frequency of 1023 mutants with a higher resistance to photo-oxidative stress compared to carotenoid-deficient mutants was observed [9]. Therefore, generation of mutations can be ruled out as a reason for increased resistance, but a frequency of 1023 mutations would only have generated a higher resistance in 0-1% of the cellular population. Singlet oxygen induces carotenoid biosynthesis in Pfaffia rhodozyma [10] and very likely mediates the light-dependent induction of carotenoid biosynthesis in Myxococcus xanthus [11]. In plants, zeaxanthin accumulates only under photooxidative stress and plays an essential role in protection of the photosynthetic apparatus via the xanthophyll cycle [12]. Therefore, we expected an increase of relative carotenoid content in R. sphaeroides under photo-oxidative stress. However, neither an increase in carotenoid contents nor increased expression of carotenoid synthesis genes was observed in R. sphaeroides. Therefore, other factors must exist in R. sphaeroides which are expressed in response to photo-oxidative stress to allow adaptation to higher singlet oxygen levels.

Protective role of carotenoids: photo-oxidative stress:

Not only does the prevention of singlet oxygen generation play a pivotal role in organisms, but so does the quenching of singlet oxygen [13, 14]. Prosthetic groups and the extension of the conjugated electron system, i.e. the structural features of carotenoids, largely influence their efficiency to quench singlet oxygen [15, 16]. Taking into account this information, the question arose whether the carotenoids found in R. sphaeroides are able to prevent damage from externally generated singlet oxygen. Water-soluble photosensitizers have been frequently used to increase the amount of singlet oxygen generated in biological systems [17]. The survival rate of all strains impaired in carotenoid biosynthesis decreased under conditions of photo-oxidative stress, indicating that quenching of singlet oxygen generated by methylene blue depends on the specific amount of carotenoid present in vivo [15]. This theory is supported by the amount of carotenoids found and the survival rate observed in strains impaired in carotenoid synthesis and wild-type cultures. Clearly, survival and carotenoid content increased in the order TC67, TC23, TC40/ TC52 and wild-type cultures. The importance of the type of carotenoid present in vivo becomes clear from the comparison of strains TC40 and TC52. Although similar amounts of carotenoids were observed, the neurosporene-containing strain (TC40) exhibited higher survival rates than the sphaeroidene-containing strain (TC52). Hence, our in vivo data are not in agreement with results obtained from in vitro systems where it was clearly observed that neurosporene is less efficient in singlet oxygen quenching than sphaeroidene [16].

Conclusions and future directions

Determining how cells sense and respond to $^1O_2$ stress is necessary for a complete understanding

of cellular survival to this toxic ROS. The recent use of traditional, high throughput and genomic approaches has greatly advanced our understanding of photooxidative stress responses in both photosynthetic and non-photosynthetic bacteria. Much work remains to be carried out to determine the mechanistic details of how cells sense, respond to and protect themselves from $^1O_2$, both in bacteria and eukaryotes.

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