Chlorophyll breakdown in aquatic ecosystems

Hugo Scheer[1]

Department Biologie I–Botanik, Universität München, 80638 Munich, Germany

The annual cycle of greening and degreening of plants is probably the most obvious sign of life on Earth. It is caused by the biosynthesis of ~10^9 tons of chlorophylls (Chls) in spring and their degradation in fall. The biochemistry of Chl breakdown to linear tetrapyrroles has been worked out in land plants (1), but little was known about the process in marine organisms, which contribute a comparable share to global biomass production. In PNAS, Kashiyama et al. (2) now provide evidence for a rather different pathway that is widespread in aquatic ecosystems and relates to protist feeding on picoplankton.

Chls are the pigments of photosynthesis, the process providing the basis for life on Earth, the oxygen in the atmosphere, and fossil fuels. These pigments have been optimized during evolution for the efficient harvesting of sunlight, and the subsequent energy transduction into high-energy compounds like ATP and NADPH. Chemically, Chls are cyclic tetrapyrroles. They share the basic carbon skeleton and large parts of their biosynthesis with hemes. The biophysical properties are nonetheless very different and reflect the different functions. Chls have, in particular, several properties that apparently render them indispensible for photosynthesis (3). They strongly absorb visible and near-infrared light, which is essential for light harvesting, and they have long-lived excited states that prevent loss of the transiently stored energy into heat.

Chls are nonetheless a mixed blessing, because the very properties beneficial to photosynthesis also render them highly phototoxic. This is no problem as long as the system works perfectly. Under optimum conditions, the primary processes, the so-called "light reactions," proceed with quantum efficiencies near the theoretical limit (4, 5). Whenever there are internal or external disturbances and the energy stored in the Chls' excited states cannot be used productively within picoseconds, these pigments can, however, raise havoc. The long-lived excited states of Chls and reverse electron flow processes now allow the formation of triplet states, which, in turn, can react efficiently with molecular oxygen. The resulting reactive oxygen species are among the most aggressive agents in nature, capable of attacking almost any cellular component. Situations generating such overload are common in a changing light environment. Photosynthetic organisms therefore need to optimize photosynthesis continuously in competition with others without being scorched by excess energy (6). Sudden and unpredictable increases can occur, for example, under moving clouds or under a canopy of other plants. They can also arise from imbalances of electron flow or lack of electron donors or acceptors caused by environmental conditions and repair of the complexes involved. Photosynthetic organisms possess a hierarchical set of efficient photoprotective systems that have coevolved with and comprise a considerable fraction of the photosynthetic apparatus. They act by preventing the formation of reactive oxygen species, as well as by detoxifying them in case the first lines of defense prove inefficient. The danger in the absence of such an effective mechanism against the photodynamic action of Chls is witnessed by their severe toxicity in animals, which is exploited in their application in cancer therapy (7).

Conversion of Chl *a* to cyclopheophorbide *a*-enol is a major detoxification pathway for protists feeding on unicellular algae.

Photodynamic damage is also a problem during Chl metabolism and the assembly, disassembly, reorganization, and repair of the photosynthetic apparatus. The biosynthetic intermediates preceding protoporphyrin IX are colorless, but all subsequent ones are colored and photodynamically active. Also, Chls are unsafe unless integrated into the photosynthetic pigment protein complexes. These processes are tightly regulated, and interception is a powerful means of weed control (6). Last but not least, the photodynamic potential of Chls becomes a problem when the photosynthetic apparatus is disassembled. Land plants have solved the problem of photodynamic damage during degreening in the fall by converting the macrocyclic Chls into open-chain tetrapyrroles. The early steps involve demetalation of the Chls and subsequent cleavage of the macrocycle at the C-5 methine bridge (Fig. 1). The resulting open-chain tetrapyrroles pose only slight photodynamic danger; they contribute to the coloration of leaves in fall; they can be diagnostic for tissue damage; and they may also be involved in other processes, including photoprotection (8).

Much less is known about Chl degradation in aqueous environments. There are scattered reports on a number of different compounds that accumulate in aging cultures of algae or phototrophic bacteria (9–11), but their significance under natural conditions has remained unclear. Protection from damage by Chls is likely to be less important for short-lived unicellular phototrophs than for perennial plants. Instead, protection now becomes a problem for organisms feeding on them. Cyclopheophorbide *a*-enol has been reported in marine animals (12, 13) and sediments (14); the study of Kashiyama et al. (2) now links its formation to protist predation. From these microscopic eukaryotes, it is further distributed along the food chain and by sedimentation processes. Evidence is provided that conversion of Chl *a* to cyclopheophorbide *a*-enol (Fig. 1) is a major detoxification pathway for protists feeding on unicellular algae and, in particular, on photosynthetic picoplankton. Although the tetrapyrrole macrocycle is retained in the process, Chl enols are nonfluorescent and nonphototoxic (2, 15, 16). They contribute up to 43% of the total chlorophyllous pigments in a water column and up to 81% in sediments, making them a major breakdown product on a global scale. It is further argued that the pigment seems to be a useful marker for herbivory by heterotrophic protists, which is difficult to assess otherwise because the majority of these organisms cannot be cultured; their diversity is derived from community sequencing (17).

The finding of such large amounts of cyclopheophorbide *a*-enol in protist metabolism poses a number of questions. The enzymology needs to be worked out; ring closure between the 17-propionic acid side chain and the isocyclic ring in the test tube requires a strong base (10, 16). It is also unclear if the protist predators produce the enzyme or if it is

Author contributions: H.S. wrote the paper.

The author declares no conflict of interest.

See companion article on page 17328.

[1]E-mail: hugo.scheer@lmu.de.

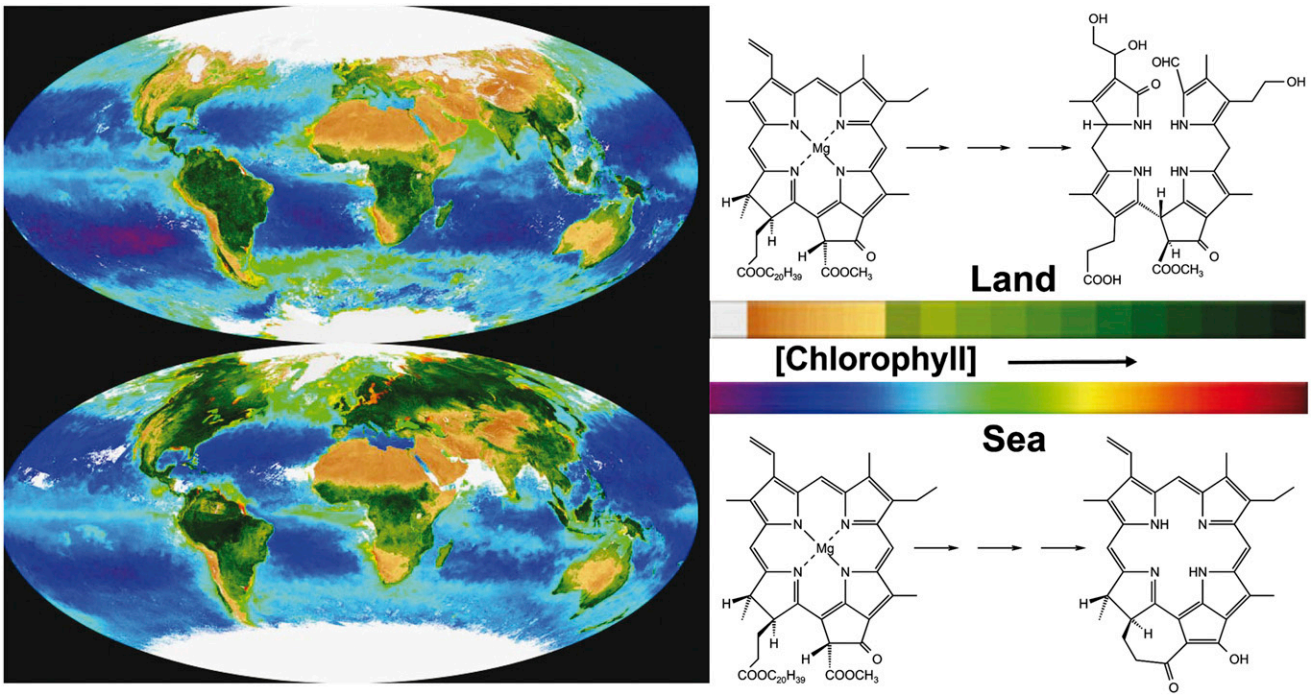

Fig. 1. Annual changes of Chl and degradation products on land and in the seas. (*Left*) Global Chl distribution in northern winter (*Upper*) and summer (*Lower*); the false-color coding scales are shown on the right for land (*Upper*) and the seas (*Lower*). White areas are due to cloud cover, ice, or, at polar winter, lack of illumination by the sun. (Images courtesy of NASA/SeaWIFS/GeoEye.) Land plants degrade Chl *a* by ring opening of the tetrapyrrole macrocycle (*Upper Right*) (1); the major breakdown product of Chls from phytopicoplankton in the aqueous habitats is cyclopheophorbide *a*-enol (*Lower Right*) (2). In both cases, the resulting products are nonphototoxic. Degradation to cyclopheophorbide *a*-enol occurs largely in heterotrophic protists after plankton ingestion.

induced in the prey, which sometimes survives for a considerable time internally after ingestion. Another question concerns the pigment structure. Although it is clearly derived from Chl *a*, *Prochlorococcus*, as one of the dominant members of picoplankton, contains Chl *b* as well (18), and both pigments carry a second vinyl group at C-8 that is absent in the product found. In phototrophic organisms, reductases are known that convert Chl *b* to Chl *a*, as well as reductases of the 8-vinyl group. Do the protists also have such enzymes, or do they use the ones from the picoplankton? In land plants, Chl *b* is converted to Chl *a* before degradation (1), but direct degradation has been reported in a green alga (9). It may also be worthwhile to check multicellular herbivores for the enzyme(s). Mammals can actively excrete Chl derivatives that have crossed the intestinal barrier (19), but there may be additional mechanisms. Last but not least, large unicellular as well as multicellular algae do not enter the food chain by protist predators. How are their Chls degraded? It seems that understanding of Chl breakdown in aqueous environments is now at the same point that breakdown in land plants was about 15 y ago, when the corner of the blanket was just being lifted.

ACKNOWLEDGMENTS. I thank Norman Kuring and Compton Tucker (National Aeronautics and Space Administration) for granting the use of the sea-viewing wide field-of-view sensor images of the Earth (January and July 1999, Hammer projection).

1. Hörtensteiner S, Kräutler B (2011) Chlorophyll breakdown in higher plants. *Biochim Biophys Acta* 1807(8): 977–988.
2. Kashiyama Y, et al. (2012) Ubiquity and quantitative significance of detoxification catabolism of chlorophyll associated with protistan herbivory. *Proc Natl Acad Sci USA* 109:17328–17335.
3. Grimm B, Porra R, Rüdiger W, Scheer H, eds (2006) *Chlorophylls and Bacteriochlorophylls: Biochemistry, Biophysics, Functions and Applications* (Springer, Dordrecht, The Netherlands).
4. Green B, Parson W, eds (2003) *Light-Harvesting Antennas in Photosynthesis* (Kluwer, Dordrecht, The Netherlands).
5. Deisenhofer J, Norris JR, eds (1993) *The Photosynthetic Reaction Center* (Academic, New York).
6. Mochizuki N, et al. (2010) The cell biology of tetrapyrroles: A life and death struggle. *Trends Plant Sci* 15: 488–498.
7. Brandis A, Salomon Y, Scherz A (2006) Chlorophyll sensitizers in photodynamic therapy. *Chlorophylls and Bacteriochlorophylls: Biochemistry, Biophysics, Functions and Applications, Advances in Photosynthesis and Respiration*, eds Grimm B, Porra R, Rüdiger W, Scheer H (Springer, Dordrecht, The Netherlands), pp 461–483.
8. Moser S, Müller T, Holzinger A, Lütz C, Kräutler B (2012) Structures of Chlorophyll Catabolites in Bananas (*Musa acuminata*) Reveal a Split Path of Chlorophyll Breakdown in a Ripening Fruit. *Chemistry* 18 (35):10873–10885.
9. Gossauer A, Engel N (1996) Chlorophyll catabolism—Structures, mechanisms, conversions. *J Photochem Photobiol B* 32:141–151.
10. Ma L, Dolphin D (1996) Synthesis and spectral studies of natural chlorins having antioxidative activity. *Pure Appl Chem* 68:765–769.
11. Schoch S, Scheer H, Schiff JA, Rüdiger W, Siegelmann HW (1981) Pyropheophytin a accompanies pheophytin a in darkened light grown cells of *Euglena*. *Z Naturforsch C* 36(c):827–833.
12. Ma L, Dolphin D (1998) Review article number 137: The metabolites of dietary chlorophylls. *Phytochemistry* 50 (2):195–202.
13. Louda JW, Neto RR, Magalhaes ARM, Schneider VF (2008) Pigment alterations in the brown mussel Perna perna. *Comp Biochem Physiol B Biochem Mol Biol* 150 (4):385–394.
14. Ocampo R, Sachs JP, Repeta DJ (1999) Isolation and structure determination of the unstable 132, 173-Cyclopheophorbide a enol from recent sediments. *Geochim Cosmochim Acta* 63:3743–3749.
15. Scheer H, Katz JJ (1975) New peripheral metal complexes related to chlorophyll. *J Am Chem Soc* 97: 3273–3275.
16. Isenring HP, et al. (1975) Enolierte Derivate der Chlorophyllreihe; 13^2-Desmethoxycarbonyl-17^3-desoxy-cyclochlorophyllid a-enol und eine Methode zur Einführung von Mg unter milden Bedingungen. *Helv Chim Acta* 58: 2357–2369.
17. Not F, del Campo J, Balagué V, de Vargas C, Massana R (2009) New insights into the diversity of marine picoeukaryotes. *PLoS ONE* 4(9):e7143.
18. Chisholm SW, et al. (1992) *Prochlorococcus marinus*, nov. gen. nov. sp., an oxyphototrophic marine prokaryote containing divinyl Chlorophyll *a* and *b*. *Arch Microbiol* 157:297–300.
19. Jonker JW, et al. (2002) The breast cancer resistance protein protects against a major chlorophyll-derived dietary phototoxin and protoporphyria. *Proc Natl Acad Sci USA* 99(24):15649–15654.