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Personal perspective

Linking the xanthophyll cycle with thermal energy dissipation*

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Abstract

This perspective summarizes my personal recollections about the initial discovery of the involvement of the xanthophyll cycle in photoprotective energy dissipation, starting with my arrival at Olle Björkman's laboratory at the Carnegie Institution and focusing on events from the mid-1980s to the early 1990s.

The beginnings

A great many people have contributed to the topic of 'Energy Dissipation and the Xanthophyll Cycle,' and there are probably as many beginnings to this story as there are contributors. One of these stories began on a beautiful spring day under the California sun in 1984, when Olle Björkman picked up his new postdoc (Barbara Demmig) from Germany, at the San Francisco Airport. Olle had just returned from a long Australian field trip where he had begun to unravel some of the secrets of the amazingly stress-tolerant mangroves – as the only trees capable of sinking their roots into saltwater-soaked sands along the world's sun-scorched tropical shores (Björkman et al. 1988).

Since I (Barbara) had an interest in halophytes as well, we were initially planning to focus our joint efforts on aspects of the salt tolerance of the mangroves. My thesis work had been on ion exchange processes across the chloroplast envelope at the University of Würzburg with Hartmut Gimmler and later also Ulrich Heber (at what was then known as Lehrstuhl I). While working with isolated spinach chloroplasts, I was often nagged by the feeling that these chloroplasts in my test tube might be doing different things when sitting in a real leaf. Since I also felt that knowing everything there was to know about spinach was still not going to reveal many fundamental physiological traits of plants growing around the globe, I was delighted to be able to also work with Klaus Winter (from the neighboring Lehrstuhl II) on the halophyte and inducible CAM (Crassulacean acid metabolism) plant *Mesembryanthemum crystallinum*, albeit still with isolated chloroplasts (for a historical minireview on CAM, see Black and Osmond, this issue). When I expressed my desire to study whole plants from a variety of different environments to visiting professor Dick Dilley (from Purdue University), he advised me to go and 'learn from the best,' who in his opinion was Olle Björkman at the Department of Plant Biology at Stanford of the Carnegie Institution of Washington. Klaus enthusiastically echoed this opinion, and after reading some of Olle's papers, I wrote a letter to Olle at the Carnegie. Everyone cautioned me not to expect too much because Olle had a reputation for, shall we say, not answering every letter he received. After some waiting time, however, a letter from Olle did indeed arrive, sent from his sabbatical leave in Australia and inviting me to join him as a postdoctoral associate.When I first arrived at the Carnegie Institute of Washington at Stanford, California, I was quite overwhelmed by all the new things with which I had absolutely no experience. The most manageable piece of equipment around was

* This paper is dedicated to my mentor and friend Olle Björkman.

Olle's 'fluorescence machine,' a custom-built low temperature chlorophyll (Chl) fluorometer that Olle had taken to the sandfly-and-crocodile-infested Australian beaches. Even though I did not have any previous experience with Chl fluorescence either, it was easy enough to open the door, stick in a sample, close the door, pour in some liquid nitrogen, turn on the light, and wait for the most amazing fluorescence wiggles to appear.

At the time, much of the focus in Chl fluorescence research was placed on following a high light-induced inactivation of Photosystem II (PS II) that was and still is commonly regarded as reflecting damage to PS II. Upon exposure to high light, the mangroves indeed exhibited a very strong quenching ('loss') of Chl fluorescence that was not readily reversible when the plants were returned to low light (Björkman et al. 1988). It was as if the entire fluorescence signal vanished before my eyes during high-light treatment, and I called this response 'the mangroves' going into hiding.' As stated before, I was painfully (or blissfully?) ignorant of anything that had been done in this area before, and furthermore preferred spending my time in front of the fluorometer rather than reading the fluorescence literature. The response of the mangroves to high light struck me as having a protective quality to it. It was also attractive to imagine mangroves in the field as possessing some powerful mechanism continually protecting them from the intense sun, rather than suffering continuous damage in their native, albeit extremely stressful habitat.

The fluorescence changes displayed by the mangroves consisted of a strong lowering of the yield of maximal Chl fluorescence (F_m) as well as a less pronounced decrease in the yield of initial Chl fluorescence (F_o). According to the ingenious model by Warren Butler (Kitajima and Butler 1975), such changes are expected as a result of increases in the rate constant for thermal energy dissipation in the light-harvesting system, K_D. Using Butler's model, Olle ascertained that the magnitude of changes in F_m and F_o could be accounted for solely by increases in K_D (Björkman 1987a). Olle and his previous postdoctoral associate from Australia, Steve Powles, had studied the response of another perennial plant native to stressful habitats with intense sunlight and intermittent severe droughts, *Nerium oleander*, to water deficits (Björkman and Powles 1984). Upon careful reexamination of the original fluorescence traces from *Nerium oleander*, Olle concluded that decreases in F_o fluorescence were present there as well (Björkman 1987b), although not as apparent as in the mangrove where the whole quenching process was more pronounced. This example illustrates the advantage in choosing suitable, extreme examples of specialization as study objects: In their fluorescence response, the mangroves disclosed the process of thermal dissipation in the light-harvesting system clearly and unequivocally – whereas it had been easy to miss in previously studied plant species.

Figure 1. Top: Barbara Demmig-Adams (left, standing) and Olle Björkman (right, standing); sitting on the ground is Olle's technician Karen Hall. *Bottom:* Barbara (left, standing) and Olle (right, standing); between them is business manager Mary Smith. Both photos were taken at Barbara's farewell party at the Carnegie Institution of Washington's Department of Plant Biology in Stanford, California, in the spring of 1986.

While I thoroughly enjoyed speculating about what might be causing the switch to effective dissipation of excess energy, I did not – as Olle accurately put it – have the faintest concrete idea as to what might be go-

ing on (Demmig et al. 1987a). Olle would caution that we might very well be on a 'wild-goose chase,' and I had to agree. While visiting my parents in Germany in the summer of 1985, I met with Engelbert Weis (University of Düsseldorf), soon to be a postdoctoral associate with Joe Berry at the Carnegie in Stanford. We poured over some of the fluorescence data Olle and I had obtained, and as we mused about what might be involved, Engelbert remarked that one might think of the xanthophyll carotenoids in this context. I told him that I had described the light-dependent conversions of the 'xanthophyll cycle' in my doctoral thesis (Demmig 1984, p. 62) – even though it really did not belong in the context of ion transport – and then the conversation drifted off to other topics. Whereas I was familiar with the principal conversions in the cycle, I could not remember seeing actual kinetics of these. Upon returning to the Carnegie, I searched through Olle's reprint collection and found papers by Hartmut Lichtenthaler (Karlsruhe) and others showing the time course of conversions in the xanthophyll cycle in high light and subsequent return to low light (Prenzel and Lichtenthaler 1982; Hager 1980). These kinetics looked the same to me as those of the K_D changes Olle and I had been characterizing (Demmig and Björkman 1987; Björkman 1987a, b). I had the intense intuitive feeling that this 'was it,' and promptly announced this to Olle. But it was not until after the excitement-filled years in Olle's lab (see Figure 1) that an opportunity presented itself to take a closer look at carotenoids upon my return to the Universität Würzburg in the spring of 1986.

It is appropriate to interrupt here with another beginning that had occurred several decades earlier. In the late 1950s, D.I. Sapozhnikov (University of Leningrad) observed a light-dependent conversion between two carotenoids in the chloroplast (Sapozhnikov et al. 1957), and assumed that the xanthophyll violaxanthin became converted to another xanthophyll, lutein. However, it was Harry Yamamoto (University of Hawaii) who established that violaxanthin is converted to zeaxanthin, and not lutein (Yamamoto et al. 1962). Harry continued with a series of groundbreaking studies on the biochemistry and regulation of this violaxanthin cycle or xanthophyll cycle (see Yamamoto 1979; see Harry's photograph in a historical article by Govindjee and Seufferheld 2002). A number of his papers ended with a note wondering about the role that these light-induced conversions might be playing in the functioning of the chloroplast.

Tackling carotenoids in Germany

Back in the year 1986, I was now a member of Würzburg's Lehrstuhl Botanik II, sponsored by Klaus Winter and Otto Lange who generously allowed me to pursue my interests. Botanik II was located next door to a Pharmaceutical Biology department, headed by Franz-Christian Czygan who had considerable experience with natural compounds, including carotenoids. It was his skilled technician, Almuth Krüger, who conducted many painstakingly elaborate analyses of my leaf extracts by thin layer chromatography. Czygan's lab had found the only commercially available thin layer plates capable of separating zeaxanthin from its close isomer lutein. This collaboration produced correlations between changes in thermal energy dissipation and increases in foliar zeaxanthin content of several perennial plant species under exposure to excess light (Demmig et al. 1987b), as well as in *Nerium oleander* exposed to a combination of high light and drought (Demmig et al. 1988). These correlations existed not only during the onset of changes in high light but also during the recovery process. After the first of these results had been published, a letter arrived from Harry Yamamoto describing his excitement about these findings and announcing that these had prompted him to return to active science after a long interlude in administration. Harry subsequently made a series of contributions to the evolving new field; many of them with his then graduate student Adam Gilmore who has also continued as an important contributor to the field that included a successful collaboration with Govindjee at the University of Illinois at Urbana (see, e.g., Gilmore et al. 1995,1998; Gilmore 1997). A special issue of *Functional Plant Biology*, edited by Gilmore et al. (2002), contains a photograph of the participants at the 'Light Stress and Meeting,' held in Herron Island, Australia.

While Harry's response was very encouraging, the same cannot be said for the responses of a majority of my colleagues. My proposal that zeaxanthin might be involved in energy dissipation received surprisingly negative comments, among which one of the less demeaning ones was the accusation to be 'breaking the laws of thermodynamics.' It was felt by many at the time that a direct energy transfer from (overexcited) chlorophyll to zeaxanthin was impossible. Higher plant carotenoids were assumed to have relevant (first singlet) excited states *higher* than those of chlorophyll, which would make the necessary *downhill* energy transfer (from Chl to zeaxanthin for energy

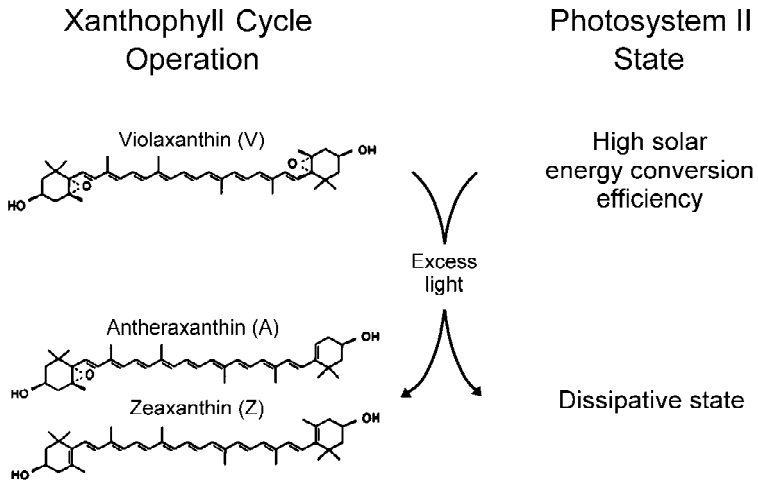

Figure 2. The three carotenoids of the xanthophyll cycle and their conversion under excess light as well as the conversion of Photosystem II from a state of high energy conversion efficiency to a dissipative state that depends on the presence of zeaxanthin plus antheraxanthin (Z + A).

dissipation) impossible. As much as this assumption was very common, it was not, however, based on any factual evidence. It was not until much later that Harry Frank in Connecticut arrived at the conclusion that the first singlet excited state of zeaxanthin does, in fact, lie below that of chlorophyll (Frank et al. 1994). To this date, more than 15 years after the original proposal, the mechanistic role of zeaxanthin in energy dissipation remains poorly understood, and direct downhill energy transfer is contemplated alongside with a possible indirect action of zeaxanthin (Demmig-Adams and Adams 1966; Demmig-Adams et al. 1996; Horton et al. 1996; Baroli and Niyogi 2000; Govindjee 2002). This lack of mechanistic understanding, however, did not slow an explosive expansion of this field in recent years that has illuminated the very important role zeaxanthin plays in the photoprotection of plants. Ironically, the perception that there was no mechanism seemed to have been the single most important factor keeping researchers from considering a role for zeaxanthin in energy dissipation – until my ignorance allowed me to consider it long enough to become taken with the idea.

Furthermore, in the late 1980s, it was felt by many in the field that the type of slowly reversible fluorescence quenching (often termed 'q_I' for inhibitory quenching), that had been examined by Olle and myself (and was expressed as an increase in the rate constant 'k_D' for thermal dissipation in the Chl pigment bed by us), was mechanistically unrelated to the widely studied rapidly reversible type of quenching (pH-dependent or energy-dependent quenching 'q_E'). This assumption was based simply and solely on the fact that the relaxation kinetics of these two events were different. While our Chl fluorescence measurements in Stanford had relied on low-temperature fluorescence, requiring several minutes of dark adaptation of leaf samples, I was able to use the room temperature fluorescence system designed by Ulrich Schreiber (Schreiber et al. 1986) for my combined fluorescence and xanthophyll measurements in Würzburg. Thus addressing the rapidly reversible, high-light component of fluorescence quenching, I went on to propose that *all* of fluorescence quenching (i.e. both 'q_E' and 'q_I'), and thus all of thermal energy dissipation, may be catalyzed by the xanthophyll cycle (Demmig-Adams et al. 1989a–c; see Figures 2 and 3). Any role of zeaxanthin in the rapidly reversible component of energy dissipation, however, had to be reconciled with the (seemingly irreconcilable) fact that the relaxation kinetics of these two processes did not match at all. Named after its most apparent feature, rapidly reversible energy dissipation/fluorescence quenching relaxed almost instantly, within a few seconds upon darkening of leaves. However, that left all of the leaf's zeaxanthin to be reconverted to violaxanthin on a much slower time scale. Nonetheless I stubbornly kept on pursuing a possible involvement of zeaxanthin in energy dissipation. I was bombarded with advice, from friends and competitors alike, to 'let it go,' to avoid hurting myself. But I had done thousands of fluorescence measurements, many by now with concomitant pigment data; and a myriad of little features just seemed to match. This feeling of seeing

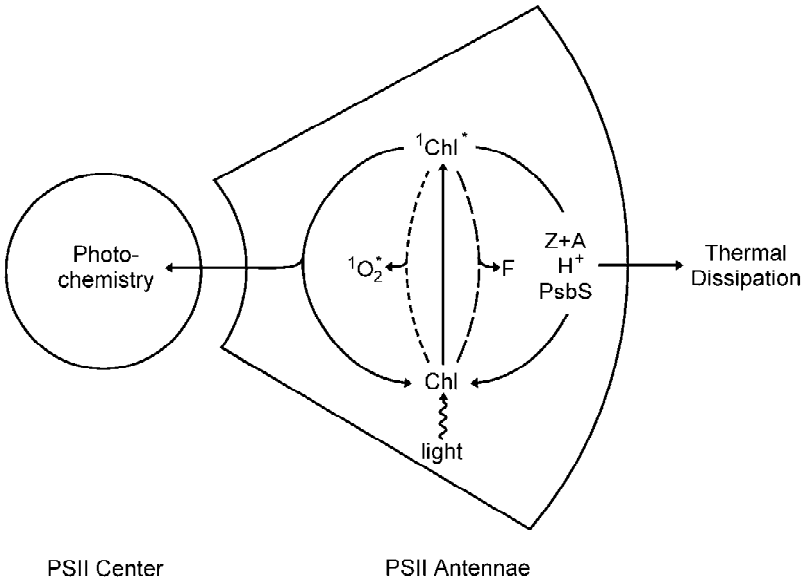

Figure 3. Four different routes of excitation energy in Photosystem II light-collecting antennae. After moving an electron of chlorophyll to the (singlet) excited state, this energy can be used either for photochemistry or, alternatively, be dissipated thermally (as heat) in a process facilitated by zeaxanthin plus antheraxanthin (Z + A), an acidic thylakoid pH, and the PsbS protein. A very small fraction of excitation energy is re-emitted as chlorophyll fluorescence (F), and can be used to monitor the fate of excitation energy. If excited singlet chlorophyll were allowed to accumulate transiently, energy could also be transferred to oxygen, forming destructive singlet excited oxygen.

patterns without being able to fully and consciously grasp them (yet) may be what intuition is, and what precedes the conscious, logical determination of fact. Surrounded by opposition, I was grateful for those few colleagues whose instincts led them to commit their labs to studying energy dissipation and the xanthophylls cycle (such as Olle, Harry Yamamoto, and also Peter Horton in Sheffield). Furthermore, their efforts made me only intensify my own since, in the event that my hypothesis should turn out to be incorrect, I certainly wished to be the one to demonstrate that too.

In a paper in 1989, I proposed that 'zeaxanthin could be related to both components [of energy dissipation] if its putative action as a quencher was controlled not only biochemically via its synthesis, but also biophysically via a second level of control in the form of high-energy-state dependent rapid activation (deactivation) step' (Demmig-Adams et al. 1989a). Straying from photosynthesis, I also suggested that 'the eyes of animals with photoreceptors present therein are probably the closest analogue to the photosynthetic membranes of plants' (Demmig-Adams 1990) and that zeaxanthin and lutein in insect and vertebrate eyes may function in photoprotection as well, possibly even via dissipation of excess energy. A role of zeaxanthin and lutein in photoprotection of human vision has now been established (Seddon et al. 1994; Chasan-Taber et al. 1999) and was recently extended to possible roles of these carotenoids in immune modulation and protection from heart disease and cancer (reviewed in Mares-Perlman 2002).

In the meantime, I had teamed up – in science and in life – with William Adams, who had come to Würzburg after studying PS II fluorescence in cacti and other CAM plants with Barry Osmond, often under extreme field conditions (Adams et al. 1987, 1988). These plants had shown F_o quenching just as pronounced as in the mangroves, leading to William's suggestion that what he was seeing was also a form of photoprotection, and he gladly joined me in my pursuit.

Continuing research in California

Meanwhile, in California's sun-flooded southern Central Valley, Olle was studying photosynthesis and fluorescence characteristics in the light in field-grown cotton with Connie Shih and Christian Schäfer (Schäfer and Björkman 1989). Olle and Sue Thayer developed an elegant HPLC (high pressure liquid chromatography) method to measure xanthophyll cycle conversions and found that sun leaves possess

much larger total xanthophyll cycle pools than shade leaves (Thayer and Björkman 1990). Olle and his next postdoctoral associate, Wolfgang Bilger (also from Würzburg), searched for ways to inhibit zeaxanthin formation in leaves to directly test the proposed involvement of zeaxanthin in energy dissipation. Using the inhibitor dithiothreitol – which had earlier been noted to inhibit the enzyme violaxanthin de-epoxidase (Yamamoto and Kamite 1972) that converts violaxanthin to zeaxanthin – they were able to show that this inhibition of zeaxanthin formation caused a major inhibition of fluorescence quenching in the light, i.e. an inhibition of energy dissipation (Bilger and Björkman 1990). This was the first evidence directly supporting a role of zeaxanthin in thermal energy dissipation. Olle and Wolfgang began computing fluorescence quenching after the Stern-Volmer equation (based on the ratio of unquenched to quenched F_m fluorescence), and referring to the entire fluorescence quenching process as NPQ for *n*on*p*hotochemical *q*uenching. They also addressed the additional condition required to engage zeaxanthin in energy dissipation and postulated that the combined presence of zeaxanthin and thylakoid acidification leads to a structural change that engages thermal energy dissipation (Bilger and Björkman 1994; see Figure 3).

Broadening the scope

By this time more and more researchers from around the world were studying energy dissipation and the xanthophyll cycle, some fascinated with, others opposed to the idea, but not too many displaying the mythical impassionate nature of scientific inquiry. William and I (Figure 4) and our many students have focused on ecological and comparative aspects of xanthophyll cycle function and acclimation in the field as well as on an integration of zeaxanthin-dependent energy dissipation into whole plant response to environmental stress (Demmig-Adams and Adams 1996, Demmig-Adams et al. 1999; Adams et al. 2001). Olle continued to study aspects of the biochemistry, physiology, and ecology of thermal energy dissipation and the xanthophyll cycle with his many associates (see special issue of *Photosynthesis Research*, Vol. 67(1–2), 2001). Most recently, an extensive collaborative effort among Kris Niyogi, Arthur Grossman, and Olle Björkman, as well as others, applying the tools of molecular biology, has brought proof of a necessary involvement of xanthophylls in energy dissipation in higher plants (Niyogi et al. 1998; Niyogi 1999) as well as the important discovery that the presence of a specialized member of the family of light-harvesting proteins is required for the structural change in the thylakoid membrane that engages rapidly reversible energy dissipation (Li et al. 2000; see Figure 3).

Figure 4. Barbara Demmig-Adams and William Adams with their children Robert and Melanie in their native Colorado in the fall of 2001.

Acknowledgments

I feel extremely fortunate for having had the opportunity to be involved in the evolution of the ideas described here and to witness their transformation into the currently accepted model for how plants protect themselves against excess light. One reason I was able to be there from the beginning is that Olle Björkman put me there. I would like to express my deepest gratitude to Olle for getting me involved in what is described in this persepctive, and for his guidance and his friendship. In addition, I thank him for his many thoughtful comments on this paper, and for giving his approval of my recollection of things. The other reason for any contributions I was able to make to the story of the xanthophyll cycle is in my thought processes. I seem to constantly make connections that often exist only in my mind. When one of these connections turns out to be true, I am at peace with myself. For everything else, I owe infinite thanks to my family for their patience with me. I am grateful to my husband, colleague, and friend William Adams for sticking with me throughout this wild and wonderful ride, as well as his comments on this paper. I would also like to thank

my children, Robert and Melanie (see Figure 4), for helping me keep my balance in life and science, and for their patience. This paper was edited by Govindjee.

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