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Chapter 1

A Random Walk To and Through the Xanthophyll Cycle

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Summary

This is an account of my personal and professional life as a student of the violaxanthin-antheraxanthin-zeaxanthin scheme for the xanthophyll cycle in higher plants. I had no early vision of becoming a scientist, but one circumstance led to another, and what began as a random walk ultimately developed into a life-long study of the biochemistry, physiology, and function of the xanthophyll cycle. The circumstances and people with whom I shared this path are described, with special attention given to the early developments.

I. Introduction

Does anyone accept an autobiographical assignment such as this without some hesitancy? I appreciated the invitation to tell my story, but wondered if I had anything worth contributing. What should I say? Who would care? After some reflection, I thought (or possibly rationalized) that my story, which is best characterized as a random walk to and through the xanthophyll cycle, may give comfort to young people whose vision of what they wish to accomplish in life may not be entirely clear. The circumstances that led to the discovery of light-induced conversions among violaxanthin (V), antheraxanthin (A), and zeaxanthin (Z)–the VAZ pathway for the xanthophyll cycle–may also be of interest. Although the walk was random, with many small and uncertain steps, it almost always carried me forward and ultimately brought me to the "right" path. As a child and even through college, I had no thoughts about becoming a scientist, only an innate desire to seek a better future as my parents had done. With luck and help from many people, I have been privileged to the better life, better than I could have imagined possible as a child. I extend my special thanks to the book's editors for giving me a chance to reflect and open doors to many good memories.

II. The Beginnings

To start, I can thank my father for my good fortune at being born in the U.S. Dad, the second eldest son, had to seek his independent fortune and immigrated to Hawaii while still a teenager. I grew up in the shadows of the famous Moana Hotel on Waikiki Beach. Perhaps some readers who have visited Waikiki remember the large banyan tree in the International Market Place located cater-corner from the hotel. We lived about three hundred yards from that tree; once, I fell out of it while playing Tarzan and broke my arm. Our home was provided by the hotel because Dad, a carpenter, was on call "24-7." We were poor, but I was not aware of it; my

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parents never complained, and all those around us were also poor. It was a happy and carefree time for me.

I was 8 years old when the Japanese attacked Pearl Harbor. Both my parents were treated as aliens even though Mom was a native-born U.S. citizen. Fortunately, we and many others in Hawaii were not sent to the "relocation camps" in which Americans of Japanese descent were detained on the U.S. mainland. In Hawaii, most of us were spared relocation largely by the actions of John Burns who, as police captain in charge of espionage for the FBI, vouched for the loyalty of Japanese-Americans in Hawaii. Burns was Delegate to Congress when Hawaii became the 50th state and later was elected Governor for three terms. He touched many lives. During the war, I carried a gas mask to school and my club house was the underground shelter Dad had built for our safety. The attack on Pearl Harbor had been led by Admiral Yamamoto; although he was no relation, I avoided problems by assuming the name Harry Chang when around soldiers on "R & R" (rest and recuperation) in Waikiki.

As a child I must have shown an interest in science because one of the best Christmas gifts I recall receiving was a Gilbert Chemistry Set. I can still picture it. It came in a red fold-out wooden case with rows of chemicals in small bottles, a simple balance, a watch glass, and spatulas. It had a manual from which I learned to make, among other things, black powder and "stink bombs." My parents weren't always pleased with the results of my experiments. These types of sets may not be available today and, if they are, their contents are probably more limited given modern concerns about hazardous substances.

III. Education

My friends are the reason I went to college. I took the entrance examination to the University of Hawaii only because they did. A few months later, I enrolled as a freshman and chose medical technology as my major because that is what a friend had selected and, much to my liking, it had a strong science emphasis. The course load was so heavy in zoology, microbiology, and chemistry that it nearly met the major's requirement for each of those fields. However, botany was not required for obvious reasons: medical technology deals with sick people, not sick plants. I didn't know then that I would spend my entire professional life happily working on plants.

The senior year in medical technology consisted of laboratory rounds at hospitals, public health laboratories, and the blood bank. During that year, I took night calls at Kuakini Hospital on alternate nights to earn my tuition for the year. Working as I did, I learned that the field of medical technology, as least at the time, offered limited economic opportunities. This important realization probably came about because by then I had a steady girl friend and was thinking about how to become a good provider. After graduating with a B.S. (1955) and completing a six-month tour of duty as a 2nd lieutenant with the U.S. Army Infantry in Ft. Benning, Georgia and Fort Riley, Kansas as part of my eight-year obligation in the Army Reserves, I embarked on the next leg of my random walk. I enrolled in the M.S. program in the Department of Food Technology at the University of Illinois at Urbana-Champaign. The selection of food technology as a field of study is not as curious as it may seem. I had worked in the Del Monte pineapple cannery for three summers prior to my senior year and was promoted each year to a better paying and more responsible position. I could see that a large food processing company offered many opportunities and thought that an advanced degree in the field would be useful. In changing to food technology, I accepted the possibility of not being able to return to Hawaii since most major food industries, except for pineapple processing, were on the mainland. It was a risk that I was willing to take. As it turned out, the greater risk was the demise of the pineapple canning industry in Hawaii, brought about by foreign competition. With one exception on the island of Maui, the canneries have all since closed.

Attending the University of Illinois was a good decision in several ways. First, I learned that, contrary to what I had assumed, changing fields of study was relatively easy. I discovered it wasn't necessary to complete all the requirements of the previous degree before starting work on a higher degree. Being the only one of three siblings to pursue graduate studies, I hadn't known any better. Next, during my first meeting to discuss my academic program with Reid Milner, Chairman of the Department of Food Technology, he casually asked if I intended to go on for the Ph.D. Me, whose parents had little schooling, who went to college only because his friends were going, and who had decided to pursue the M.S. only as a means for gainful employment? It was an unexpected and welcome expression of confidence in my potential. Thank you Prof. Milner! Finally, while pursuing the M.S., I found that I was more interested in "Why?" than "How?" and preferred fundamentals to applications. The title of my M.S. thesis was "*Kinetic Studies on the Heat Inactivation of Peroxidase in Sweet Corn.*" Peroxidase activity was used, and is

possibly still being used, as an indicator for adequacy of heat treatment (blanching) of corn prior to freezing. Blanching prevents frozen corn from developing undesirable "off" flavors that result from enzyme activity. I found that there were two types of peroxidases with markedly different heat sensitivities (Q_{10}). The inactivation of both forms followed pseudo first-order kinetics, and the stability of the heat-resistant component made heating to inactivate it almost futile. I think it was this study that awakened by interest in basic science. I have the University of Illinois, and the Department of Food Technology in particular, to thank for giving some direction to my random walk.

The University of California, Davis had a program that seemed ideal for me: a Ph.D. in Comparative Biochemistry within the Department of Food Science and Technology. I was married and had a young son by then. The three of us drove for California, pulling our worldly possessions in a U-Haul trailer. We felt like pioneers traveling cross-country with an infant, but instead of in a covered wagon, we had an aging Ford. In Lincoln, Illinois, just a hundred miles out of Champaign-Urbana, our car broke down and required, so we were told, a complete engine overhaul that strained our resources and delayed our journey by several days. Even with the repairs, we barely made it over Donner Pass at the border between Nevada and California. A few months ago we drove through that region and the incline was hardly noticeable. Was the U-Haul so heavy, the road now less steep, or the rental car that much better? Perhaps yes to all. Thinking back on it now, that trip in the summer of 1958 was a great adventure and a test of endurance. What better preparation could one have for a doctoral program?

At Davis, my research advisor was Clinton "Chi" Chichester, a student of Gordon Mckinney, both of whom were interested in the biosynthesis of carotenoids. Paul Stumpf was my academic advisor. The biosynthetic pathway for carotenoids was not yet clearly established. During my first year I worked on an early step in the pathway and published a note in *Nature* (Yamamoto et al., 1961).

IV. The VAZ Pathways Story

I owe much of what came next to Sputnik, the first satellite, which was placed into orbit by the Soviet Union in October 1957. This achievement shocked the U.S. into giving more support to science and not just to space science. I benefited from this new commitment through a National Science Foundation predoctoral fellowship that funded the balance of my doctoral program. Besides relieving me of financial worries, the fellowship allowed me more flexibility in selecting a research topic. Also as a result of Sputnik, English translations of Russian articles became available, and a paper by David I. Sapozhnikov was brought to my attention by Tommy Nakayama, a friend and colleague of Chi's (Fig. 1). Sapozhnikov et al. (1957) reported that, in leaves subjected to alternating light and dark treatments, high light induced reciprocal changes in the levels of violaxanthin and lutein. He hypothesized that the reaction was involved in photosynthetic "oxygen transfer," that is, from water to molecular oxygen. I believed the observation merited further study because, unlike most carotenoids that are metabolic end products, this system appeared to be dynamic. Also, if the cycle was indeed involved in photosynthetic oxygen evolution, it would be a very significant discovery. I felt, however, that the reported kinetics made this possibility unlikely. Furthermore, if the reaction was involved in oxygen evolution, it was not an essential pathway since oxygen-evolving organisms such as blue-green algae lack violaxanthin.

Fig. 1. Photograph taken at the National Science Foundation sponsored Carotenoid Symposium in Kyoto, Japan, 1965. Clinton Chichestor is being greeted by Prof. H. Mitsuda. Tom Nakayama is in the background.

Of course, the instrumentation and analytical methods available 45 years ago were crude by today's standards. I used preparative columns packed with powdered sugar to separate the xanthophylls of saponified extracts of leaves. Saponification removed chlorophyll that these columns could not resolve from xanthophylls. To assure complete recovery of xanthophylls after saponification, the xanthophylls were washed into ethyl ether instead of petroleum ether. Safety precautions were not what they are today and I was lucky not

to have blown up the lab and myself with it. I would often return to my apartment reeking of ether. The procedures were so slow that I could obtain barely two sets of data points in a day. Despite these limitations, the very first experiment confirmed that high light induced in leaves a decrease in violaxanthin and an apparently corresponding increase in lutein. However, I was still not fully convinced that a symmetrical reactant, violaxanthin, was being converted to an asymmetrical product, lutein. Two mono-de-epoxidase reactions by different enzymes could explain such a conversion but would not be consistent with Sapozhnikov's hypothesis, which implied a single-step double de-epoxidation. Alternatively, the product could be zeaxanthin rather than lutein, leaving open the possibility of a single-step double de-epoxidation. Since the sugar column resolves pigments by normal-phase partitioning, I reasoned that zeaxanthin would, if formed, likely co-migrate with lutein, given that both molecules have similar structures and identical numbers of hydroxyl groups. Rechromatography of the sugar column's lutein band on a magnesium oxide (adsorption) column showed the product to be zeaxanthin and not lutein. The next question presented itself: does the conversion to zeaxanthin occur in one step or two? Antheraxanthin, the expected product of a two-step reaction, was found on the sugar column as a faint band between violaxanthin and lutein. These results established the currently accepted VAZ scheme: the light-induced cyclical conversion, in leaves, of violaxanthin (V) through antheraxanthin (A) to zeaxanthin (Z) (Yamamoto et al., 1962). Today, the pathway for the cycle seems obvious and can be easily demonstrated by HPLC analyses with a column that has mixed partitioning and absorption properties (Gilmore and Yamamoto, 1991). I referred to the pathway as the "violaxanthin cycle" but now "*the* xanthophyll cycle" is more commonly used. I emphasize "the" to acknowledge that other xanthophyll cycles are known, specifically the diadinoxanthin cycle in several algal species (Hager and Stransky, 1970) and the lutein epoxide cycle in mistletoe (Matsubara et al., 2001). It is uncertain whether these other xanthophyll cycles have the same biochemistry and functions as the VAZ cycle.

The VAZ scheme, in my opinion, was strong evidence against Sapozhnikov's hypothesis. Besides the very slow kinetics and incorporation of ^{18}O from O_2 on re-epoxidation of zeaxanthin (Takeguchi and Yamamoto, 1968), stepwise mono-de-epoxidation excluded a single-step removal of the epoxides of violaxanthin as might be expected for a role in oxygen evolution. Prof. Sapozhnikov acknowledged that the product of violaxanthin was zeaxanthin but, as far as I am aware from the literature, he ignored antheraxanthin and continued to suggest a role for the cycle in oxygen evolution (Sapozhnikov, 1973). Regrettably, I did not get to meet Prof. Sapozhnikov to congratulate him for his initial observation that light induces a change in the violaxanthin concentration. Following the VIIIth International Congress on Photosynthesis, which was held in Stockholm, Sweden in 1989, I went on a tour to the Soviet Union. In St. Petersburg I met a few of Prof. Sapozhnikov's former associates and learned that he had passed away in Italy in 1985 on his way to his new adopted home in Canada. Olga Koroleva gave me a photograph of his group taken in 1974; it is a wonderful photograph and I share it as a tribute to him (Fig. 2). In a different vein, I also express my appreciation to C. Stacy French, who was Director of the Carnegie Institution of Washington (Stanford, California), for his encouragement, patience, and graciousness to an aspiring graduate student. I visited his laboratory several times to learn as much as I could about photosynthesis and became friends for life.

V. Further Adventures and Advances

The notion that pursuing higher education would preclude my returning to Hawaii proved wrong. A year before completing the Ph.D., I was offered and accepted a position in the newly formed Department of Food Science and Technology in the College of Tropical Agriculture at the University of Hawaii. By then I had two children, and a secure job was attractive. For the first few years, I pursued research related to agriculture and refrained from working on the xanthophyll cycle, expecting that another student in Chi's lab would take up the work. When it became clear that no one would, I returned to the xanthophyll cycle, focusing on the biochemistry with the long-range objective of gaining insights into function. During my xanthophyll cycle hiatus (1962–65), Achim Hager made significant progress on the cycle's biochemistry. He showed that violaxanthin de-epoxidase (VDE) was localized in the chloroplast lumen and required ascorbate and low pH for activity (Hager, 1966). The cycle's transmembrane organization (Fig. 3) was established when both groups showed that the reverse epoxidation of zeaxanthin to violaxanthin occurred on the stromal side of the thylakoid at near neutral pH in the presence of NADPH and O_2 (Hager, 1975; Siefermann and Yamamoto, 1975).

Working on the xanthophyll cycle in Hawaii was not easy. Funding was limited, and there were no researchers nearby with whom I could interact that were engaged in related work on photosynthesis or carotenoid biosynthesis. Fortunately, grants from the

Fig. 2. 1974 photograph of David I. Sapozhnikov's group given to me by O. Koroleva when I visited St. Petersburg in 1989. Front row from left: I. Popova, D. I. Sapozhnikov, S. Eidelmann, O. Popova. Back row from left: E. Morkovskaja, M. Gabr, O. Koroleva, T. G. Maslova, and G. Kornjushenko.

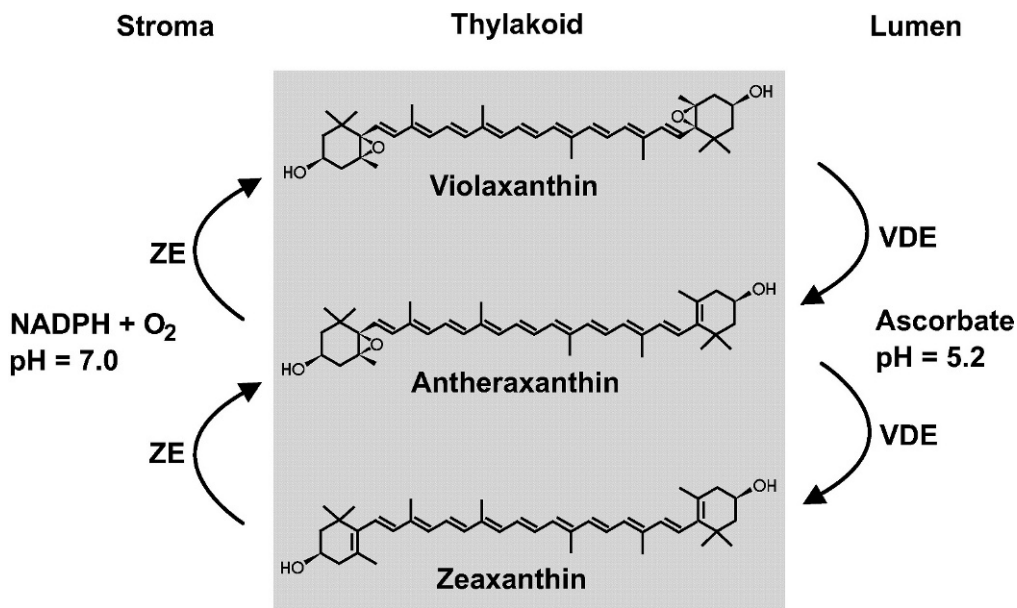

Fig. 3. VAZ transmembrane pathway for the xanthophyll cycle in higher plants.

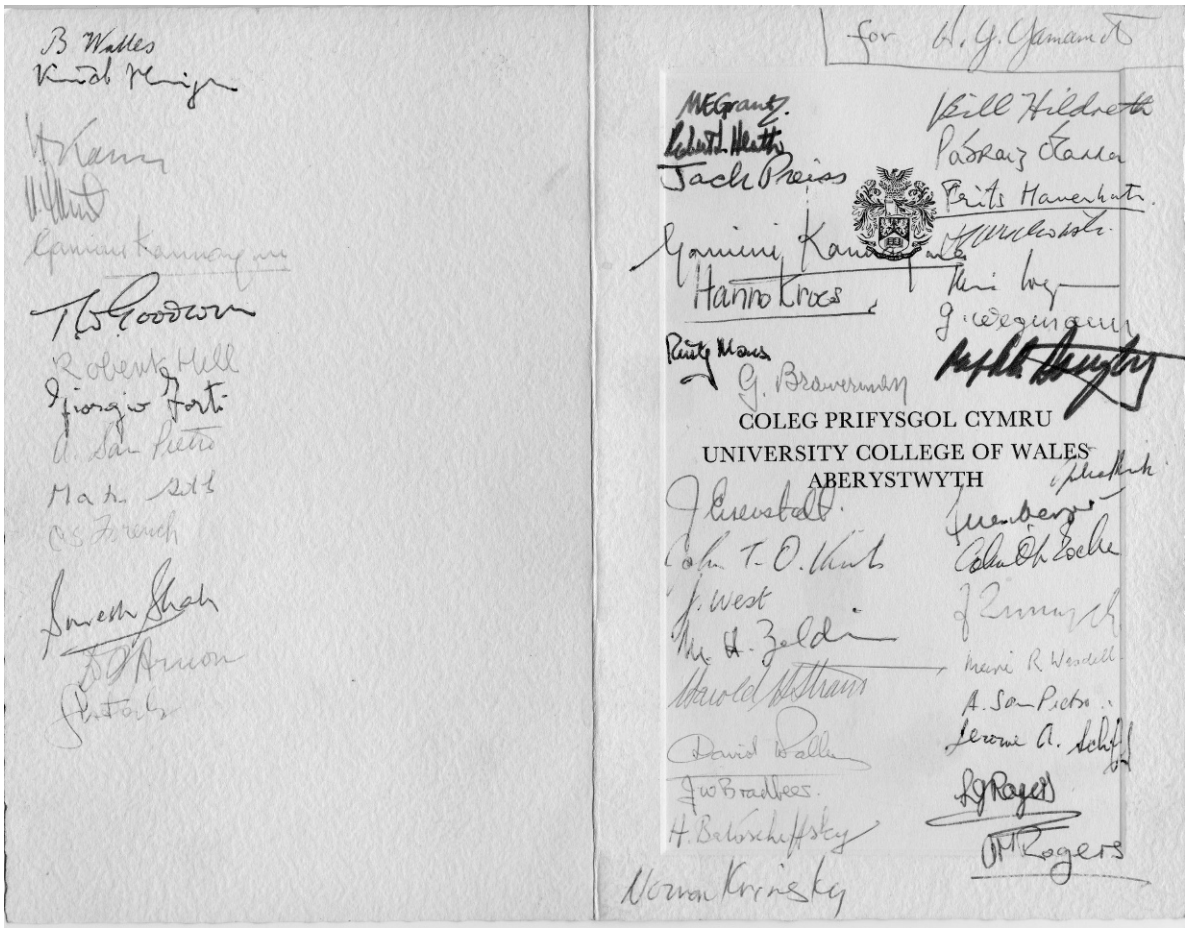

Fig. 4. Autographed banquet menu from the 1965 NATO Advanced Study Institute on the Biochemistry of Chloroplasts in Aberystwyth, Wales. In addition to names already mentioned, signatures by Trevor Goodwin, Giorgio Forti, Martin Gibbs, Norman Krinksy, Harold Strain, Jack Pries and Joseph Bradbeer, among others, are also present. How many signatures can you, the reader, recognize? It was exciting for me to be at this meeting of such notable scientists, most of whom I met for the first time.

National Science Foundation, the U.S.D.A. Competitive Grants Program, and the Department of Energy allowed me to continue research on the VAZ cycle. These grants also enabled me to travel about once a year to a major meeting on photosynthesis. Given my isolation from the mainstream of photosynthesis research, the importance of attending these meetings cannot be overemphasized. The first international meeting I was privileged to attend was the Advance Study Institute on the Biochemistry of Chloroplasts held in Aberystwyth, Wales in 1965, sponsored by the North Atlantic Treaty Organization (NATO). I believe my invitation to attend came from Trevor W. Goodwin. While looking through memorabilia in preparation for this perspective, I found the menu that I had passed around for signatures at the farewell dinner meeting (Fig. 4). I hope readers can make out the names in this marvelous collection of signatures. Among them are Robin Hill, Tony San Pietro, C. Stacy French, Dan Arnon, and many more, with apologies to those I have not mentioned.

Contact with the photosynthesis and plant biochemistry community has been an essential part of my forty-year stroll through the xanthophyll cycle and has created opportunities I might otherwise have missed. For example, in 1968 I spent my first sabbatical with Leo Vernon at the C.F. Kettering Research Laboratory in Yellow Springs, Ohio. There I met Teruo Ogawa, who was completing a postdoctorate with Leo, and with whom I became close personal friends. Teruo introduced me to the "opal glass" spectrophotometric technique perfected by Kazuo Shibata for measurement of light-scattering samples (Shibata, 1973). Upon returning to Hawaii, I applied the technique to chloroplast suspensions and found that violaxanthin de-epoxidation was detectable as a difference spectrum, with a peak at 505 nm, and could also be followed

kinetically at 505 *minus* 540 nm (Yamamoto et al., 1972). This sensitive and rapid method for *in situ* measurement of xanthophyll cycle activity in chloroplasts was key for much of the progress we made during the 30 years that followed. Early applications of the spectrophotometirc assay included the discovery of the "availability" phenomenon, the intensity-dependent fractional release of violaxanthin from the total pool (Siefermann and Yamamoto, 1974); inhibition of VDE by dithiothreitol (Yamamoto and Kamite, 1972); and epoxidation of zeaxanthin to violaxanthin (Siefermann and Yamamoto, 1975). The method was also well suited for *in vitro* studies that demonstrated the requirement of lipid for de-epoxidation of pure violaxanthin (Yamamoto et al., 1974) and the substrate stereospecificity of VDE (Yamamoto and Higashi, 1978). The spectrophotometric assay of VDE activity remains useful to this day. It was recently applied to demonstrate that monogalactosyldiacylglycerol (MGDG), the major thylakoid membrane lipid, has a limited capacity to accommodate zeaxanthin and when this capacity is exceeded, stereospecific product feedback inhibition of VDE results (Hieber et al., 2004).

The serendipitous discovery of MGDG as the optimal chloroplast lipid for *in vitro* de-epoxidation of violaxanthin proved important. While I was able to obtain de-epoxidation of violaxanthin bound in washed thylakoid membranes, the same crude VDE preparation had no activity against purified violaxanthin, as had been reported by Hager (1966). Violaxanthin is insoluble in aqueous buffer, and various attempts to suspend or solubilize violaxanthin in a form that yielded activity failed. Isomerization and decomposition of the preparation were excluded as possible reasons. In the course of these tests, I ran out of the violaxanthin preparation that I had been using and, as a matter of convenience, recovered violaxanthin from "fat plates"* that Dorothea Siefermann, then a postdoctoral researcher in my laboratory, happened to be using for analysis of chloroplast pigments. Violaxanthin that was eluted from these plates with acetone and used without further purification gave rapid and nearly complete conversion to zeaxanthin. The reason for this success was traced not to coconut oil from the plates but rather to a lipid component in the unsaponified extract that co-chromatographed with violaxanthin. C. Freeman Allen earlier had separated the lipids in chloroplasts (Allen et al., 1966) and he kindly sent me samples that he still had on hand. All of the lipid samples we received supported de-epoxidation to varying degrees. We subsequently prepared a complete set of the major chloroplast lipids and found that MGDG was the most effective, giving rapid and complete de-epoxidation of violaxanthin in about 5 minutes under optimal conditions (Yamamoto et al., 1974). These results helped define the *in vivo* substrate of VDE: the violaxanthin that is converted to zeaxanthin is free in the membrane lipid phase rather than bound to pigment proteins. Exchanges between protein-bound pigments and free pigments in the lipid phase are implied. Recently, model systems consisting of soybean phosphatidylcholine only (Grotz et al., 1999) or egg phosphatidylchloline combined with MGDG (Latowski et al., 2002) have confirmed that lipid is required for "activation" of pure violaxanthin. However, de-epoxidation in these presumably bilayer systems were relatively slow and incomplete compared to de-epoxidation in the MGDG micelle system. MGDG constitutes a much larger fraction of the total chloroplast lipid: 60% to phosphatidylcholine's 2% or less (Webb and Green, 1991) and thus the micelle system may more closely approximate the *in situ* environment of free violaxanthin. Whatever model system is employed, violaxanthin should be prepared from saponified extracts to avoid artifacts from even trace amounts of contaminating chloroplast lipid.

We used the pH-dependent binding of VDE to the thylakoid membrane and to MGDG to obtain the partial C-terminal sequence (Rockholm and Yamamoto, 1996), which was then used to clone the gene and express the VDE protein (Bugos and Yamamoto, 1996). The complete sequence showed that VDE was a lipocalin enzyme, the first identified in plants** (Bugos et al., 1998; Yamamoto et al., 1999). This finding confirmed conclusions drawn 20 years earlier–before lipocalins were known to exist–that the shape of the VDE active center resembled a deep well (Yamamoto and Higashi, 1978). The cloned VDE carried out the forward VAZ reaction, providing strong evidence that the reaction could be catalyzed by a single enzyme with mono-de-epoxidase function. Evidence that a single gene product accounted for de-epoxidation was shown by Niyogi et al. (1998), in which a deletion mutation in *Arabidopsis* inhibited all de-epoxidase activity. The cysteine rich domain in the N-terminal sequence and highly charged domain in the C-terminal sequence explained, respectively, the DTT inhibition (Yamamoto and Kamite, 1973) and the pH-dependent membrane binding of VDE (Rockholm and Yamamoto, 1996).

My walk through the xanthophyll cycle took several administrative detours from 1980–82, 1982–86, and 1994–96 as Acting Associate Dean of Research, Chair of the Department of Plant Molecular Physiology, and Director of the Hawaii Agricultural Experiment Station, respectively. During the second of these, another chance occurrence caused me to refocus on

research. It was popular for a time to hold small, informal bi-national conferences in Hawaii. One such conference, on photoinhibition, was held in Honolulu in 1985. At that time the subject was outside of my field of interest, but I attended on invitation from David Fork, whom I knew from visits to Carnegie during my days as a graduate student. One report by Olle Björkman caught my attention. He showed the kinetics of chlorophyll fluorescence quenching resulting from photoinhibition, which I recognized as being similar to the kinetics of violaxanthin de-epoxidation. After the meeting, I wrote a research proposal, including a request for a pulse-amplitude modulated fluorometer (PAM)*** that I would need to investigate the possible connection between photoinhibition and zeaxanthin formation. The grant proposal was successful but I was "scooped" by publication of a seminal paper by Demmig et al. (1987) that reported the correlation between non-photochemical quenching (NPQ) and zeaxanthin formation. Barbara Demmig had, in fact, noted the possible correlation a few years earlier but had difficulty convincing others of its reality. (For an interesting account of the events surrounding her important discovery, see Demmig-Adams, 2003.) Later, Adam Gilmore showed by a modeling technique that antheraxanthin also contributed to NPQ as effectively as zeaxanthin (Gilmore and Yamamoto, 1993). It is now common practice to express de-epoxidation as the de-epoxidation state (DES), or $(Z + A)/(V + A + Z)$, in conjunction with NPQ. The question of whether the correlation is a direct or indirect effect was recently answered with evidence that zeaxanthin is a direct quencher of excess energy (Ma et al., 2003).

Advances in research often result from the coupling of new analytical instrumentation or methods with the efforts of talented and dedicated individuals. This is certainly the case for contributions my laboratory made regarding the xanthophyll cycle and its relationship to NPQ. In terms of technology, the 505-nm absorbance change associated with de-epoxidation, the MGDG model system, and the HPLC method for resolution of zeaxanthin and lutein made significant differences in our research. The 505-nm change and development of the PAM provided an exceptional opportunity to examine xanthophyll cycle activity and NPQ simultaneously in chloroplasts. This application made it possible to show that although de-epoxidation and NPQ were both induced by light-dependent low pH, the protons for each were localized in different domains of the membrane (Mohanty and Yamamoto, 1996). I have not understood why the relatively simple opal-glass technique for the 505-nm change has not found more use, especially since it can be used simultaneously with NPQ measurement. In contrast, the HPLC method we developed for analysis of plant pigments is in wide use today (Gilmore and Yamamoto, 1991). As with the identification of the 505-nm change and the development of the MGDG model system, we arrived at this method somewhat by circumstance. Thayer and Björkman (1990) had reported an HPLC method that separated lutein and zeaxanthin but the column they used was no longer available. Based on my previous experience in separating lutein and zeaxanthin by sequential partitioning and absorption columns, we looked for a column that had both of these properties. ODS-1 was identified as a possibility because of its light carbon loading and non-endcapping of active silyl groups. The column performed as we hoped.

Although the mechanism of quenching has largely been resolved, numerous questions about the xanthophyll cycle remain. The physiology of the cycle is not well understood. The pool size of violaxanthin and the fraction of the pool that is active in the cycle vary among plant species and growth conditions (Demmig-Adams et al., 1999, this volume). There is a growing body of evidence that the cycle's operations may be related to more than just NPQ (Yokthongwattana and Melis, this volume). If the cycle has multiple functions, how are these functions regulated? Mutant studies suggest that the cycle in not essential for photosynthesis (Jung and Niyogi, this volume) and yet, as far as I am aware, all wild-type plants have the xanthophyll cycle. Why has nature retained this complex, apparently multifunctional system if it is not of some critical advantage? Did the system provide the adaptability to light environments needed for terrestrialization over multiple generations? Is it simply coincidental that the dominant photosynthetic life forms in the ocean and on land have xanthophyll cycles, the diadinoxanthin cycle and the VAZ cycle, respectively? The xanthophyll cycle has been related to photoprotection of plants against sudden and prolonged light stress (Verhoeven et al., 2001) and to improved plant fitness, as indicated by seed production, under fluctuating light intensities of the natural environment (Külheim et al., 2002). Interestingly, only half of the VAZ cycle (to antheraxanthin) appears to be present in a few Rhodophyceae (Aihara and Yamamoto, 1968) and in *Mantoniella* (Goss et al., 1998). Are these species less fit? We recently proposed that zeaxanthin functions as a messenger in a signal-transduction network that operates in the lipid phase of the chloroplast membrane to explain the cycle's multifunctional capabilities (Hieber et al., 2004). As one who has been involved with the xanthophyll cycle for nearly 45 years, I am surprised at how the questions seem never to end.

Fig. 5. Millie and I seated for lunch in Bagan during a recent tour of Myanmar.

VI. Many Thanks to Many

The cycle was the vehicle through which I entered the world of photosynthesis, traveled world wide, and made many good friends. I thank the photosynthesis community, the granting agencies, students, postdoctoral researchers, and colleagues for making my journey such a joy. I have also been blessed with recognition from peers through two awards, the Samuel Cate Prescott Award for Research in 1969 from the Institute of Food Technologists and the Charles Reid Barnes Life Membership Award in 2003 from the American Society of Plant Biologists. I extend special thanks to Govindjee, who offered me encouragement at every stage. Most importantly, I thank my family, especially my wife, Millie, for being understanding and supportive of my "obsession" for these many years. Now that I am retired, we have more time to spend together (Fig. 5). The question that I asked so many years ago as to why higher plants have retained the cycle remains unanswered. To all who will be continuing the walk, I look forward to learning what you find. I hope you enjoy the journey as much as I have!

Notes

*Fat (Egger) plates are Kieselguhr G plates that are dipped in hydrogenated coconut-oil/hexane solution and dried prior to use (Egger, 1962; also see Yamamoto, 1985). The plates, equivalent to a C_{18} endcapped HPLC column, resolve chlorophylls and carotenoids, except for lutein from zeaxanthin, in unsaponified chloroplast extracts. Inasmuch as lutein concentration is not normally affected by light treatments, Egger plates are an inexpensive method for tracking xanthophyll cycle activity.

**Lipoclains are a family of proteins that transport small, hydrophobic molecules such as retinol and porphyrins.

***I met Ulrich Schreiber, the developer of the PAM, at the 1971 International Congress of Photosynthesis meeting in Stresa, Italy. He approached me to discuss my paper on the incorporation of ^{18}O from O_2 into antheraxanthin and violaxanthin (Takeguchi and Yamamoto, 1971). I always appreciated his expression of interest, which was offered long before much was known about fluorescence quenching, let alone its relationship to the xanthophyll cycle.

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