Photosynth Res (2007) 92:145–148
DOI 10.1007/s11120-007-9196-7

INTRODUCTORY ARTICLE

# Thioredoxin: an unexpected meeting place

**Bob B. Buchanan**

Received: 24 January 2007 / Accepted: 13 May 2007 / Published online: 19 July 2007


**Abstract** For much of the latter part of the 20th century, photosynthesis research at Berkeley was dominated by Daniel Arnon and Melvin Calvin. In this article, I have briefly described how their contributions jointly provided the foundation for our work on thioredoxin and how important Andrew Benson was to this effort.

**Keywords** Andrew A. Benson · Daniel I. Arnon · Melvin Calvin · Roger Y. Stanier · Reductive carboxylic acid cycle · Reverse citric acid cycle · *Chlorobium thiosulfatophilum* · *Chlorobium limicola*

The contributions of Daniel Arnon and Melvin Calvin to photosynthesis at Berkeley are legendary. And so they were in 1962—the year of my arrival on the campus as a postdoc in the Department of Biochemistry. I knew neither individual at that time, but, due to unforeseen events, I joined the Arnon laboratory the following year, September 1963 (Buchanan 2001; http://www.nap.edu/readingroom/books/biomems/darnon.html).

At the time of my arrival in the Arnon laboratory, Arnon and Calvin operated independently, and there was little interaction between the two. This situation endured despite the fact that their research was complementary. In fact, as seen below, earlier findings of the two laboratories were brought together by our own work on thioredoxin.

Prior to my joining the Arnon laboratory (September 1, 1963), Roger Stanier of the Department of Bacteriology, organized several joint meetings of the two groups to exchange ideas. In addition to Stanier, participants included Arnon and Calvin, themselves, as well as members of their groups. However, as several of the sessions proved overly fractious, the meetings were short-lived (Roderic Park, a member of the Calvin group at the time, recounts his experience at one of the sessions.[1] Further interaction during the 1960s was confined to occasional individual discussions of Arnon and Calvin with younger scientists interested in photosynthesis. However, even these meetings were rare, and in some cases, were actively discouraged by the leaders of the laboratories (an example of these meetings is described[2] by

B. B. Buchanan (✉)
Department of Plant and Microbial Biology,
University of California, 111 Koshland Hall,
Berkeley, CA 94720, USA
e-mail: view@nature.berkeley.edu

[1] I remember the abortive attempts by Roger Stanier to reconcile the Calvin/Arnon axis well! At their very first meeting I was called upon as a young postdoctoral student (leading lambs to the slaughter) to present some work I was involved in at that time. This first seminar was held in the old Life Sciences Building in early 1959 I believe. About 10 min into the talk Calvin and Arnon started arguing about relative reaction rates and their consequences on the abundance of $^{14}C$ labeled metabolites in the chromatograms we were viewing. I stopped speaking as the two of them continued to argue, Calvin with the precision of a physical chemist and Arnon with the reasoning of a philosopher. After 5 min or so Arnon made an analogy by comparing the reaction under discussion to ''a man with an athletic heart.'' Calvin interrupted ''to hell with athletic hearts, let's get back to photosynthesis!'' By this time the room had gone silent and everyone had forgotten what I was talking about! This was my introduction to academic debate at Berkeley. (R. P. Park February 20, 2007).

[2] In 1960 (when I was on sabbatical) the Royal Society held a garden party in Cambridge as part of their tercentenary anniversary. I was visiting Robin Hill at the time, and he took Jean and me along. He said Calvin would be there, and I told him he would have to introduce me. Every time I met Calvin in Berkeley I had to be formally introduced supposedly because I was with Arnon, and he made it a point of honor not to recognize members of the Arnon group. So, at the Garden Party I was duly introduced by Robin Hill, and, to my complete astonishment, Calvin immediately replied, ''Oh yes, I know Whatley.'' We did not talk science as this was a social occasion, but Mrs. Calvin declared that she had in her Berkeley garden an avocado tree that produced useful fruit even in a cool climate and would we like a cutting. On my return to Berkeley I visited Calvin in his lab and again had to be introduced! I suppose I was now under the patronage of Arnon rather than Hill and that made all the difference. Incidentally, we never got our avocado cutting! (F. R. Whatley March 3, 2007).

Robert Whatley, a former member of the Arnon group). One can get a sense of the atmosphere on the campus at the time[3] from Hartmut Lichtenthaler, a postdoc in Calvin's group. There was, in fact, little interchange between the groups until 1970 when John Olson, a sabbatical visitor from Brookhaven National Laboratory, started a joint photosynthesis seminar that went on for several years. Arnon (but not Calvin) was a regular participant in this weekly event that provided an excellent forum for mutually beneficial discussions. There were, however, few research interactions, and the groups remained largely self-contained.

When I joined the Arnon laboratory, my work focused on anaerobic bacteria—first on a heterotroph (*Clostridium pasteurianum*) and then on a photosynthetic organism (*Chlorobium thiosulfatophilum*, now called *Chlorobium limicola*), the latter suggested by Roger Stanier. These efforts led to the elucidation of a role for ferredoxin in $CO_2$ fixation (Bachofen et al. 1964) and the reductive carboxylic acid cycle (Evans et al. 1966)—a pathway now usually called the reverse citric acid (or reverse/reductive TCA) cycle. The new Chlorobium pathway, which was worked out jointly with Michael Evans, Daniel Arnon and myself, was the first described as being independent of the cycle identified by Calvin and collaborators for algae and leaves (Bassham et al. 1954). As detailed by Ormerod (2003), the work went firmly against entrenched dogma, and, as a consequence, almost 25 years elapsed before the cycle was accepted by the scientific community (Buchanan and Arnon 1990).

The acceptance of the work was likely held back by the fact that Calvin, himself, did not believe the evidence for the cycle (Ormerod 2003). However, in the end, other studies, including genome sequencing, confirmed the original observations: the reverse citric acid cycle was the major mechanism of carbon fixation in Chlorobium and the organism lacked the cycle described by the Calvin laboratory (Buchanan and Sirevåg 1976; Sirevåg and Buchanan 1977; Fuchs et al. 1980a, b; Ivanovsky et al. 1980; Tabita 1988; Buchanan and Arnon 1990; Eisen et al. 2002; Ormerod 2003). According to recent evidence, the reverse citric acid cycle functions more broadly than originally envisaged and is present in a variety of nonphotosynthetic (chemoautotrophic) bacteria, including magnetotactic cocci (Williams et al. 2006), and inhabitants of hydrothermal vents (Takai et al. 2005; Hügler 2007; and references therein). Oceanic vents and other warm habitats seem to be a particularly rich source for organisms using the reverse citric acid cycle. A strain of *Chlorobium* was recently identified among the inhabitants isolated from the East Pacific Rise—an area of high-volcanic activity with vents that support a variety of ecosystems (Beatty et al. 2005). In view of the diversity of microorganisms, it is perhaps not surprising that, since the *Chlorobium* work, paths of authotrophic carbon dioxide assimilation have been identified that function independently of either the Calvin cycle or the reverse citric acid cycle (Ormerod 2003).

The findings with *Chlorobium* led us to examine chloroplasts for the presence of the ferredoxin-linked carboxylases (Buchanan et al. 2002). Although these experiments were negative, they led by accident to the finding that ferredoxin, reduced photochemically by chloroplasts, activates fructose 1,6-bisphosphatase (Buchanan et al. 1967), an enzyme of the cycle of Calvin and collaborators. During this same period, J. A. Bassham, a member of the Calvin group, provided kinetic evidence that fructose bisphosphatase and several other enzymes of photosynthetic carbon dioxide assimilation are activated by light in algal cells (Bassham 1971). However, aside from a book chapter published jointly with Bassham (Bassham and Buchanan 1982), there continued to be little interaction at the level of the laboratory between the two photosynthesis groups at Berkeley.

In experiments with chloroplasts, we demonstrated relatively early that reduced ferredoxin did not interact with fructose bisphosphatase directly, but that a protein fraction was required (Buchanan et al. 1971). Due to collaborative efforts with Peter Schürmann and Ricardo Wolosiuk, 10 years after the original findings we were able to identify the two active components in that fraction (Wolosiuk and Buchanan 1977): (1) thioredoxin, a small disulfide protein that was discovered as a hydrogen donor in the reduction of ribonucleotides to deoxyribonucleotides in *E. coli* (Laurent et al. 1964), and (2) ferredoxin–thioredoxin reductase, a previously unknown iron–sulfur enzyme catalyzing the reduction of thioredoxin by ferredoxin via a mechanism unique to biology (Dai et al. 2000, 2007). Shortly thereafter, three additional regulatory enzymes of the chloroplast carbon cycle were found to be regulated by thioredoxin–phosphoribulokinase, NADP-glyceraldehyde

[3] The altercations between Melvin CaIvin with Daniel Arnon were well-known throughout the scientific community. They lead to awkward situations even when European visitors came to Berkeley. So it was when Wilhelm Menke, a pioneer in photosynthesis research, came to Berkeley from Cologne, Germany, in spring 1963. He visited Melvin Calvin in his office in the morning, and in the afternoon walked down to Calvin's laboratory in the Life Science Building to have discussions with several members of his group. Menke was interested in our work on thylalkoid lipid composition of spinach chloroplasts since my Nature paper on this topic (with Rod Park) had just appeared. When I asked Menke after the discussion about his further plans of the day, he hesitated at first and then told me that he had also an appointment with Daniel Arnon. However, he quickly added that I should not tell anyone as he did not want Calvin to learn that he would see Arnon. Furthermore, Arnon should not know that he had seen Melvin Calvin first. When I told him that I knew Daniel Arnon well and that we had had several discussions on vitamin K1 function in photosynthesis, he accepted my invitation to accompany him to Arnon's laboratory. (H. K. Lichtenthaler, April 11, 2007).

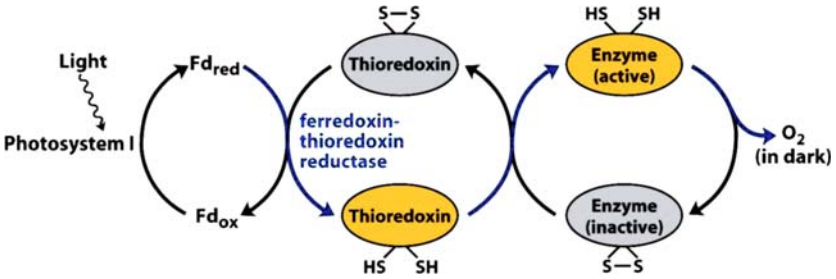


**Fig. 1** The ferredoxin/thioredoxin system of oxygenic photosynthesis. As discussed in the text, this regulatory mechanism builds on the long-term contributions of the Arnon laboratory (ferredoxin and noncyclic electron transport) and the Calvin laboratory (the photosynthetic carbon cycle). Enzymes actived in the light via ferredoxin are deactivated in the dark via oxygen. While there is oxygen uptake in the light via thioredoxin, the system is not a significant contributor to the Mehler reaction (Miginia-Maslow et al. 1984). Reproduced with permission from Lehninger, Principles of Biochemistry, 4th Edition, by D. L. Nelson and M. M. Cox, W. H. Freeman Co., New York, 2004

3-phosphate dehydrogenase and sedoheptulose bisphosphatase (Buchanan et al. 2002). The thioredoxin experiments clarified the nature of the chloroplast sedoheptulose 1,7-bisphosphatase and showed it differed from the more completely studied fructose bisphosphatase enzyme (Breazeale et al. 1978). In this way our studies contributed in a small measure to the enzymology of $CO_2$ assimilation in chloroplasts—specifically to a reaction that grew out of pioneering work of Benson et al. (1952). Several years after these four enzymes were identified as thioredoxin targets, a fifth member of the cycle was shown to be linked to thioredoxin—i.e., ribulose 1,5-bisphosphate carboxylase or Rubisco. In this case, thioredoxin did not interact with the enzyme directly, but did so by activating the associated chaperone-like protein, Rubisco activase (Portis 2003). Subsequent work has revealed that the chloroplast thioredoxin system, now generally known as the ferredoxin-thioredoxin system (Fig. 1), is present in all oxygenic photosynthetic organisms examined and apparently accompanies the capability for light-dependent oxygen evolution (Schürmann and Jacquot 2000; Buchanan and Balmer 2005). Further, a number of investigations, most recently based on proteomic approaches, have shown that the system links ferredoxin to the regulation of enzymes of a spectrum of chloroplast processes and responds to signals in addition to light—e.g., reactive oxygen species (ROS) (Buchanan and Balmer 2005).

There is one aspect of our work that stands out in relation to the early history of photosynthesis research at Berkeley. This centers on a finding made on the regulation of $CO_2$ assimilation—i.e., that thioredoxin serves as a regulatory link between ferredoxin, a focus of research in the Arnon laboratory for more than three decades (http://www.nap.edu/readingroom/books/biomems/darnon.html; Arnon 1988) and enzymes of the chloroplast carbon cycle—the pathway worked out by Calvin and collaborators in the 1950s (Bassham et al. 1954). Thus, thioredoxin builds on two of the major contributions of these laboratories in a way that highlights the importance of each. However, although both knew the work, I am not sure that either Arnon or Calvin realized that thioredoxin serves as a meeting place for their contributions.

During the course of the chloroplast work, I became aware of the contributions of Andy Benson and how critical his efforts had been to uncovering the carbon cycle—work prerequisite to our later regulatory studies with chloroplasts. For much of this period, I interacted closely with Dan Arnon who had the highest opinion of Andy. As I learned more about Andy's photosynthesis contributions and his later brilliant accomplishments on plant lipids and marine phytoplankton, I concurred heartily with Dan's opinion. And as I later came to know him personally, my admiration grew and Andy became a model as a scientist and a person. Andy, warmest congratulations on your 19th birthday!

**Acknowledgments** I wish to thank Professors Hartmut Lichtenthaler, Richard Malkin, Roderic Park, Kenneth Sauer, Achim Trebst and Robert Whatley for helpful comments on this manuscript.

## References

Arnon DI (1988) The discovery of ferredoxin: the photosynthetic path. Trends Biochem Sci 13:30–33

Bachofen R, Buchanan BB, Arnon DI (1964) Ferredoxin as a reductant in pyruvate synthesis by a bacterial extract. Proc Natl Acad Sci USA 51:690–694

Bassham JA (1971) Control of photosynthetic carbon metabolism. Science 172:526–534

Bassham JA, Benson AA, Kay LD, Harrris AZ, Wilson AT, Calvin M (1954) The path of carbon in photosynthesis. 21. The cyclic regeneration of carbon dioxide acceptor. J Am Chem Soc 76:1760–1770

Bassham JA, Buchanan BB (1982) Carbon dioxide fixation pathways in plants and bacteria. In: Govindjee (ed) Photosynthesis,

development, carbon metabolism and plant productivity. vol 2. I. II, Academic Press, NY, pp 141–189

Beatty JT, Overmann J, Lince MT, Manske AK, Lang AS, Blankenship RE, Van Dover CL, Martinson TA, Plumley FG (2005) An obligately photosynthetic bacterial anaerobe from a deep-sea hydrothermal vent. Proc Natl Acad Sci USA 102:9306–9310

Benson AA, Bassham JA, Calvin M, Hall AG, Hirsch HE, Kawaguchi S, Lynch V, Tolbert NE (1952) The path of carbon in photosynthesis. XV. Ribulose and sedoheptulose. J Biol Chem 196:703–716

Breazeale VD, Buchanan BB, Wolosiuk RA (1978) Chloroplast sedoheptulose 1,7-bisphosphatase: evidence for regulation by the ferredoxin/thioredoxin system. Z. Naturforsch. 33c:521–528

Buchanan BB (2001) Daniel I. Arnon 1910–1994. biographical memoirs 80. National Academy of Science Press, Washington DC

Buchanan BB, Arnon DI (1990) A reverse KREBS cycle in photosynthesis: consensus at last. Photosyn Res 24:47–53

Buchanan BB, Balmer Y (2005) Redox regulation: a broadening horizon. Ann Rev Plant Biol 56:187–220

Buchanan BB, Kalberer PP, Arnon DI (1967) Ferredoxin-activated fructose diphosphatase in isolated chloroplasts. Biochem Biophys Res Commun 29:74–79

Buchanan BB, Schürmann P, Kalberer PP (1971) Ferredoxin-activated fructose diphosphatase of spinach chloroplasts: resolution of the system, properties of the alkaline fructose diphosphatase component, and physiological significance of the ferredoxin-linked activation. J Biol Chem 246:5952–5959

Buchanan BB, Schürmann P, Wolosiuk RA, Jacquot JP (2002) The ferredoxin/thioredoxin system: from discovery to molecular structures and beyond. Photosyn Res 73:215–222

Buchanan BB, Sirevåg R (1976) Ribulose 1,5-diphosphate carboxylase and *Chlorobium thiosulfatophilum*. Arch Microbiol 109:15–19

Dai S, Friemann R, Glauser DA, Bourquin F, Manieri W, Schürmann P, Eklund H (2007) Structural snapshots along the reaction pathway of ferredoxin:thioredoxin reductase. Nature (in press)

Dai S, Schwendtmayer C, Schürmann P, Ramaswamy S, Eklund H (2000) Redox signaling in chloroplasts: cleavage of disulfides by an iron–sulfur cluster. Science 287:655–658

Eisen JA, Nelson KE, Paulsen IT, Heidelberg JF, Wu M, Dodson RJ, Deboy R, Gwinn ML, Nelson WC, Haft DH, Hickey EK, Peterson JD, Durkin AS, Kolonay JL, Yang F, Holt I, Umayam LA, Mason T, Brenner M, Shea TP, Parksey D, Nierman WC, Feldblyum TV, Hansen CL, Craven MB, Radune D, Vamathevan J, Khouri H, White O, Gruber TM, Ketchum KA, Venter JC, Tettelin H, Bryant DA, Fraser CM (2002) The complete genome sequence of *Chlorobium tepidum* TLS, a photosynthetic, anaerobic, green-sulfur bacterium. Proc Natl Acad Sci 99:9509–9514

Evans MCW, Buchanan BB, Arnon DI (1966) A new ferredoxin-dependent carbon reduction cycle in a photosynthetic bacterium. Proc Natl Acad Sci USA 55:928–934

Fuchs G, Stupperich E, Eden G (1980b) Autotrophic $CO_2$ fixation in *Chlorobium limicola*. Evidence for the operation of a reductive carboxylic acid cycle in gorwing cells. Arch. Microbiol 128:64–71

Fuchs G, Stupperich E, Jaenchen R (1980a) Autotrophic $CO_2$ fixation in *Chlorobium limicola*. Evidence against the operation of the Calvin cycle in growing cells. Arch Microbiol 128:56–63

Hugler M, Huber H, Molyneaux SJ, Vetriani C, Sievert SM (2007) Autotrophic $CO_2$ fixation via the reductive tricarboxylic acid cycle in different lineages within the phylum Aquificae: evidence for two ways of citrate cleavage. Environ Microbiol 9:81–92

Ivanovsky RN, Sintsov NV, Kondratieva EN (1980) ATP-linked citrate lyase in the green sulfur bacterium, *Chlorobium limicola* forma *thiorsulfatophilum*. Arch Micorbiol 128:239–241

Laurent TC, Moore EC, Reichard P (1964) Enzymatic synthesis of deoxyribonucleotides. 4. Isolation and characterization of thioredoxin, the hydrogen donor from *Escherichia coli* B. J Biol Chem 239:3436–3444

Miginiac-Maslow M, Jacquot JP, Droux M (1984) Light dependent activation of NADP-malate dehydrogenase and photophosphorylation: energy requirements. In: Sybesma C (ed) Advances in photosynthesis research. vol 3. Martinus Nijhoff/Dr. W. Junk, The Hague, pp 697–700

Ormerod J (2003) 'Every dogma has its day': a personal look at carbon metabolism in photosynthetic bacteria. Photosyn Res 76:135–143

Portis AR (2003) Rubisco activase—Rubisco's catalytic chaperone. Photosyn Res 75:11–27

Schürmann P, Jacquot JP (2000) Plant thioredoxin systems revisited. Ann Rev Plant Physiol Mol Biol 51:371–400

Sirevåg R, Buchanan BB, Berry JA, Troughton JM (1977) Mechanisms of $CO_2$ fixation in bacterial photosynthesis studied by the carbon isotope fractionation technique. Arch. Microbiol. 112:35–38

Tabita FR (1988) Molecular and cellular regulation of autotrophic carbon dioxide fixation in microorganisms. Microbiol Rev 52:155–189

Takai K, Campbell BJ, Cary SC, Suzuki M, Oida H, Nunoura T, Hirayama H, Nakagawa S, Suzuki Y, Inagaki F, Horikoshi K (2005) Enzymatic and genetic characterization of carbon and energy metabolisms by deep-sea hydrothermal chemolithoautotrophic isolates of Epsilon-proteobacteria. Appl Environ Microbio. 71:7310–7320

Williams TJ, Zhang CL, Scott JH, Bazylinski DA (2006) Evidence for autotrophy via the reverse tricarboxylic acid cycle in the marine magnetotactic coccus strain MC-1. Appl Environ Microbiol 72:1322–1329

Wolosiuk RA, Buchanan BB (1977) Thioredoxin and glutathione regulate photosynthesis in chloroplasts. Nature 266:565–567