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Personal perspective

Personal recollections of 40 years in photosynthesis research*

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Abstract

The major theme of my work in photosynthesis has been electron transport in green plant thylakoids. In particular, we investigated the properties and the role of the NADP-reducing flavoprotein and its possible function in cyclic electron transport, the regulation by protons of electron transport, and the redox system of ascorbate and monodehydroascorbate (the ascorbate free radical). The function of this system in providing ATP in the stoichiometric amount needed for carbon assimilation, and the regulation of the alternative transfer of electrons to NADP and to the ascorbate free radical were among the achievements of my collaborators and myself. Specifically, the early conviction that cyclic phosphorylation was essential part in photosynthesis was shattered as far as higher plants are concerned, and replaced by a modified Mehler reaction providing additional ATP to run the Calvin cycle. The situation seems to be different in unicellular green algae, where quantitatively much larger changes of the relative size of Photosystem (PS) I and PS II antennae during the so-called 'state' transitions have been reported, and these seem to be associated with a high activity of cyclic electron transport in state 2. Beyond the science, the friendly interactions with so many persons around the world sharing my interest in photosynthesis and in other aspects of human life have been most rewarding.

Abbreviations: AFR – ascorbate free radical; ASC – ascorbate; DCMU – 3-(3,4dichlorophenyl)-1,1 dimethylurea; NADP – Nicotinamide adenine dinucleotide phosphate; PGA – 3-phosphoglyceric acid; 1,3 bisPGA – 1,3 diphosphoglyceric acid; P700 – the reaction center of Photosystem I; PS I and PS II – Photosystem I and II, respectively; FNR – ferredoxin-NADP oxidoreductase; LHC II – light harvesting complex II, the chlorophyll a,b-protein antenna complex of PS II

First steps in science

My first interest in basic science was energy metabolism, and an important element stimulating it was reading, in 1953, a very valuable book 'Dynamic Aspects of Biochemistry', by Ernest Baldwin. I was a student of the Faculty of Agriculture in Milano, a faculty very much oriented towards practical problems rather than basic science. I was in the third year, and it was impossible to change my thesis work (an experimental work of one to two years was required, and still is, to obtain any university degree, corresponding more or less to what is called a Master's degree in some countries). Thus I had to forget about the energetics of glycolysis and respiration, and continued working on the mineral nutrition of crops fertilized with dirty waters. Nevertheless, I kept my mind and my readings oriented towards energy metabolism.

After graduating, and after a short stay in Paris working on the post-harvest respiration of bananas during fruit ripening, I was fortunate to have the opportunity to work at the Botanical Institute of the University of Milano with Erasmo Marrè, a plant physiologist, who was biochemically oriented. Marrè was an 'immigrant' to plant physiology from the med-

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ical school. I met Marrè in a very informal way, and we discussed glycolysis and the Krebs cycle; he told me his ideas about the biochemical events possibly involved in the regulation of respiration and glycolysis in plants. At the end of the conversation, he said: "Well, I see that we have the very same interests, it would be great if we could work together!" When I told him that I was looking for a job, a difficult sport in Italy in those days, he arranged matters, with the help of the director of the Institute, Prof. Sergio Tonzig, a botanist who understood that the new approach to understand the physiology of plants (in the early 1950s) should necessarily include the knowledge and methods of cell biochemistry and physiology. With that in mind, he decided to introduce Marrè into the Botanical Institute, and helped all of Marrè's efforts to set up a modern laboratory of plant physiology. I happened to be included in that program, and I became one of Marrè's collaborators in his long-term project on metabolic reactions to auxin. My first enterprise was to show that auxin treatment of pea internode segments, while stimulating growth and respiration, increased the ratio of ATP/ADP in the tissues (Marrè and Forti 1958). I measured ATP and ADP using enzymatic methods in tissue extracts, and I enjoyed adapting the methods to our instrumentation facilities. In those days (1955–1956) most of the enzymes had to be prepared rather than purchased. We also demonstrated that, *in vivo*, the auxin-stimulated respiration (only ca. 25–30% in pea internode segments) was mediated by cytochrome oxidase (Marrè et al. 1960). I studied the properties of mitochondria isolated from pea stems, and so I learned how to measure ATP synthesis coupled to electron transport. The attempts to show an influence of auxin on isolated mitochondria were, however, unsuccessful.

The 1955–1958 period of work with Marrè was very important to me, as the first real experimental investigation of a long range problem. Besides, his human qualities were such that a durable friendship between the two of us followed.

At the end of 1958, I had the opportunity to go to work with André T. Jagendorf at the Johns Hopkins University, in Baltimore. I took that opportunity, and since then, photosynthesis became my field of work, though not my only scientific interest.

Photosynthesis

I was very pleased with the friendly hospitality of André Jagendorf (see Jagendorf 1998). In his laboratory I had the choice of working on several different projects. André was very liberal about that, and I finally chose to look into the role of ascorbate in photosynthetic electron transport: it was not at all established, at that time, that there should be one. Robin Hill's scheme for photosynthetic electron transport became known to us more than one year later, and we were still talking about XH and YOH as the primary reductant and oxidant produced by the 'light reaction' of photosynthesis (see Rabinowitch 1945). We found that ascorbate strongly stimulated photophosphorylation in the absence of any added electron transport cofactor (a condition that was defined at that time as 'endogenous' photophosphorylation), and that O_2 was required for photophosphorylation under these conditions and for its stimulation by ascorbate (Forti and Jagendorf 1961). In fact, it was the first demonstration that the Mehler reaction was coupled to ATP synthesis. This concept appears obvious in today's thinking since the Mehler reaction (Mehler 1951) requires electron transport from water to O_2 through the two light reactions in series. This interpretation, however, was uncertain at the time of our experiments. We also discovered that cyanide inhibited the ascorbate effect on photophosphorylation, and that in the presence of cyanide, ascorbate was rapidly oxidized to dehydroascorbate in the light. These observations could only be interpreted correctly many years later, after the discovery of superoxide dismutase in thylakoids (Asada et al. 1974), of ascorbate peroxidase (Miyake and Asada 1992), as well as of the rapid reduction of the ascorbate free radical at the reducing side of Photosystem I, in competition with NADP (Forti and Ehrenheim 1993).

At Johns Hopkins, Anthony San Pietro and his collaborators (D.L. Keister A. and F.E. Stolzenbach) were working on the mechanism of NAD(P) reduction in chloroplasts, and the roles in it of what is now called ferredoxin, and of the flavoprotein, later called FNR (ferredoxin-NADP reductase). It is interesting to note that at that time A. San Pietro had used the term 'photosynthetic pyridinenucleotide reductase' for ferredoxin; further, it became clear that this was the same electron carrier that Robin Hill had earlier defined as methemoglobin reducing factor (see Fry and San Pietro 1963). On the other hand FNR, mentioned above, was defined, at that time, as

'NADPH transhydrogenase' because of its transhydrogenase activity (see San Pietro 1961). It was later shown that this was only an accessory activity considerably lower in terms of electron transfer rate than the main activity of the enzyme, namely, the reduction of NADP in oxygenic photosynthesis.

While in Baltimore, I had the opportunity to meet Bessel Kok, who was working on Photosystem I (PS I); Martin Gibbs, working on carbon metabolism, and many others. Travelling around the United States, to participate in meetings or to give seminars, I had the opportunity to become acquainted with many of the scientists of the photosynthetic establishment.

Most interesting to me was the symposium 'Light and Life', my first important international meeting, organized at Johns Hopkins University (see McElroy and Glass 1961). The meeting covered different aspects of photochemistry and photobiology, including light emission from fireflies, vision, and photosynthesis. The so-called Z-scheme of R. Hill was presented at that meeting, with a clear indication of the roles of the b and f cytochromes (previously discovered, also by R. Hill), which was independently proven, as far as cytochrome f was concerned, by the elegant experiments of Duysens and collaborators (1961).

When I came back to Milano, at the end of 1960, Marrè had been appointed Professor of Plant Physiology (see Marrè 1991), and in 1961 I became an assistant professor. However, we did not work together anymore, except for a very short time on the utilization of ATP produced in the light for metabolic work other than photosynthesis (Marrè et al. 1962). He pursued his studies on auxin and the regulation of metabolism and growth, and I kept working on photosynthesis, alone. The director of the Institute Prof Tonzig (a man of my father's generation) provided most of the funding for our research, and though keeping himself informed on what was going on in the laboratory, he never interfered with our projects. He was a very reserved and silent person, and his communication with the people working in the Institute was mostly through Marrè. One of his worries was that we, the young people who were biochemically oriented, would forget about the physiology of the plant and concentrate on the properties of this or that enzyme, "exchanging the means for the ultimate goal". On the occasions when I had the opportunity to talk to him, for example when crossing paths in the corridors of the Institute, I always found a liberal, helpful person: his ironic (also strongly self-ironic) smile was something that one cannot forget, and made me wonder if I had not neglected something very important.

Cyclic photophosphorylation

I easily found students willing to work with me: among them was Maria Luisa Bertolé who investigated the occurrence of cyclic photophosphorylation *in vivo*. Cyclic photophosphorylation was known to occur in isolated chloroplasts (or thylakoids), but required the addition of non physiological electron carriers, the most active of them being phenazine-methosulphate (PMS), a dye rapidly reduced by PS I and reoxidized in the intersystem chain, or directly by $P700^{+}$. Many other redox dyes catalyze cyclic photophosphorylation, provided they have the right redox potential and can carry protons across the thylakoid membrane during electron transport. Cyclic photophosphorylation was defined as phosphorylation requiring only the PS I light reaction, and therefore resistant to the herbicide diuron (DCMU). *However, no direct evidence was available at that time (nor is today!) that cyclic phosphorylation is a physiological phenomenon, essential for photosynthesis to proceed*. We decided to demonstrate it *in vivo*, by measuring the light dependent changes of ATP in leaves made anaerobic in the dark to prevent respiratory ATP synthesis, and treated with DCMU to inhibit linear electron transport. We indeed observed light dependent ATP formation (Forti and Parisi 1962). Later, Tanner and Kandler (1969) made similar observations as indicated by measuring glucose incorporation into starch (an ATP dependent reaction) in unicellular algae. They were also able to measure the quantum yield of the reaction. But in our case, as in their conditions, the PS I dependent ATP formation was the only choice left to the poor poisoned cells: again, the evidence was still insufficient to show that cyclic electron transport is part of steady-state oxygenic photosynthesis *in vivo*, under physiological conditions. At that time I became convinced that the answer was yes, and I started looking for the physiological components of the 'cyclic' electron transport system. Daniel Arnon was convinced that ferredoxin is the catalyst of cyclic electron transport around PS I coupled to phosphorylation (see the review by Arnon 1977), in spite of the fact that the rates observed in isolated chloroplasts were rather low.

Ferredoxin-NADP Reductase (FNR): Linear and cyclic electron transport and photophosphorylation

We had started an investigation (with M.L. Bertolé and B. Parisi initially, then with E.P. Sturani and G. Zanetti) of the functions of FNR, which is the same FAD flavoprotein as the NADPH diaphorase of Avron and Jagendorf (1956) and the transhydrogenase of San Pietro and coworkers (San Pietro 1961): in other words, the NADP reducing enzyme of thylakoids (San Pietro 1961). FNR, bound to thylakoids (Forti et al. 1963) or purified and soluble (Zanetti and Forti, 1966; Forti and Sturani, 1968), had NADPH-cytochrome f reductase activity. This activity was a good candidate for the cyclic electron transport: however, we only observed it upon addition of pure cytochrome f to the thylakoid suspension. We were not equipped to measure the endogenous components, and the measurement would anyhow be very difficult technically, and ambiguous to interpret.

When I was invited by the organizers (André Jagendorf and Bessel Kok) to participate in the Virginia meeting at Airlie House, in the fall 1963, I reported some of these results on FNR as a cytochrome f reductase. That meeting was memorable both for the broad field covered and for the informal and free running discussions (Kok and Jagendorf 1963). There, I heard for the first time Horst Witt presenting his results and scheme of electron transport in the form of 'rolling circles' of redox reactions following the primary photochemistry, on both sides of PS II and PS I, and Arnon lecturing with his finger shaking in front of the audience, the way an angry father does when he scolds his rebel child. Figure 1 shows some of the participants at this Congress.

At that meeting, André, Bessel, and Hans Rurainski invented an after dinner joke, in which I happened to have a major role: regally dressed, with a crown on my head, I was sitting on a throne, the throne of the 'King of the Chloroplasts', and the major photosynthetists kneeled in front of me, and kissed my regal ring. I had not been pre-informed of anything, and when Bessel, with a powerful loudspeaker, called Giorgio Forti, 'fortissimo', on the stage, I did not really understand what was going on, but it took me a millisecond to realize that, as we say in Italy, when you are invited to a dance, you must dance. As a matter of fact, I took a particular pleasure to keep down, on their knees in front of me, a few strong personalities whom I won't name. In good humor, prizes were distributed: Arnon received an arrow and a small ball; Eugene Rabinowitch received a scroll for being the prophet of photosynthesis, etc.

The work on the enzymology of FNR was pursued (with Emmapaola Sturani and Giuliana Zanetti, who both became, several years later, Professors of Biochemistry in the Faculty of Milano), and later experiments were done to prove the involvement of FNR in cyclic photophosphorylation, showing that an antibody against the pure enzyme inhibited DCMU resistant photophosphorylation in chloroplasts, broken hypotonically in the reaction medium, which obviously also contained the endogenous ferredoxin. The thylakoid bound enzyme was titrated with the antibody raised against it, and all its activities (NADP photoreduction, diaphorase, cytochrome f reductase) were inhibited as was the 'endogenous' photophosphorylation (Forti and Zanetti 1969; Forti and Rosa 1972). However, the reconstitution of NADP photoreduction upon the addition of purified FNR (of the same catalytic activity, on the basis of FAD content, as the thylakoid native enzyme) required a lot more enzyme than that originally present in the membranes, and could never attain the rates of NADP reduction of the original thylakoids. Obviously, the membrane bound enzyme was for some reason catalytically more effective. It was ruled out that interaction with ferredoxin was the cause of this, because the enzyme, whether thylakoid bound or in solution, had the same affinity constant for ferredoxin (Forti and Bracale 1984) in the formation of the binary complex which was previously demonstrated by Foust et al. (1969).

The second International Congress on Photosynthesis in Stresa, Italy

At the First International Congress on Photosynthesis in Freudenstadt, in 1968, the assembly of the participants took a vote on the site for the next Congress, three years later, and on the person who should organize it. A rather large majority voted for Italy, and for me. I suppose that the prospect of visiting Italy on the occasion of the Congress had a strong influence on that vote. I was at the same time pleased and deeply worried, knowing the difficulties in finding financial support in Italy for such an enterprise, and especially considering that I only had a very small group of collaborators. Aside from myself, there were only two on the staff in the Plant Physiology group at the University of Bari, where I became Professor of

Figure 1. A photograph of several scientists attending the 1963 meeting at Airlie House in Warrenton, Virginia. Andre Jagendorf is seated on the left in the front row. Above him is the Nobel-laureate James Franck (who was the only one spared from kneeling before me, and was not part of the joke). The author is seated in the second row, third to the right of Eugene Rabinowitch (in glasses and striped tie); Antony San Pietro is on the extreme right in the first standing row; on his right is Horst Witt; followed by Louis NM Duysens; and third from his right is Daniel Arnon, looking majestically to his right. Jack Myers is to the right and behind Ellen Weaver (white dress); to Jack's right is Bessel Kok (in glasses). Photograph is a courtesy of Govindjee.

Plant Physiology in 1967. When I moved to Napoli, in 1969, the number grew to three, but still we were a small team for organizing an international congress expected to be attended by a large number of participants. I dropped the idea of having the Congress in the Royal Palace in Napoli, an imposing and beautiful site. The large number of authorisations to be asked of the local administration and the Arts Department of the government made the bureaucratic difficulties too cumbersome. I decided therefore to run the Congress in Stresa, a touristic resort on the Lago Maggiore, near the Swiss border, easy to reach from everywhere in Europe. The date of the Congress was coincident with the second centenary of the discovery of Photosynthesis by Joseph Priestley in 1771: Robin Hill was the speaker who gave the historical account of that memorable event (Hill 1972). Figure 2 shows Robin Hill and myself at that session; please note that I am holding Robin's famous briefcase. The Congress was finally organized with the help of my wife, Annalucia, and of the photosynthetic group in Bologna: Assunta Baccarini-Melandri, Andrea Melandri, and Paolo Pupillo. I had met the Melandris (Assunta and Andrea) and Pupillo in Bologna a few years earlier, when I was lecturing in plant physiology in that University, while still working in Milano. We started a cooperation which, though limited by the fact that we were living and doing most of our work in different cities, was nevertheless fruitful and was at the origin of bringing Andrea and Assunta to move as postdoctoral collaborators to Indiana University, with A. San Pietro and H. Gest, respectively.

One of the results of the cooperation with the Melandris was some work on the development of the photosynthetic apparatus in greening bean leaves (Melandri et al. 1967). I wanted to learn more in that field, and a few years later I went to work, on a short sabbatical, with Itzhak Ohad at the Hebrew University in Jerusalem, on the development of the photosynthetic apparatus in *Chlamydomonas reinhardtii y-1*. There I met an Australian postdoctoral, Robert Jennings, one of the collaborators of Ohad's project. Jennings was just finishing his two years work in Jerusalem, and he decided to come to work in my

Figure 2. Robin Hill and the author at the 1971 Second International Congress of Photosynthesis, held in Stresa, Italy. The author is holding Robin's famous and old briefcase at the time of Robin's lecture on Joseph Priestley and his discoveries. Photograph is a courtesy of Govindjee.

laboratory, in Napoli first, then in Milano where I returned in the fall of 1974. Today, Jennings is a Professor of Photobiology in Milano. In the early eighties he started a completely independent research line, more strictly biophysical, on the transfer and trapping of excitation energy in the two photosystems, which has been going on ever since.

Structure and function of thylakoids: Regulatory phenomena

My cooperation with R Jennings lasted about 8 years, and involved also F.A. Garlaschi, who became an Assistant Professor in Napoli, and is now Associate Professor of Botany in Milano. They both followed me to Milano, where we worked together on chlorophyll *a* fluorescence changes connected with the stacking-unstacking of the membranes, reversibly induced by cations (Jennings and Forti 1974a; Jennings and Forti 1978). This was an area that was being extensively studied in many laboratories (e.g., P Homann; Govindjee; J Barber; E Gross, and many others). Also, we contributed to research on the localization, on the different regions of the membranes, of the components of the electron transport system (Jennings et al. 1981). We have shown that FNR is located outside the grana partitions, as it reacts with its antibody, a 7-S γ globulin, at the same rate in stacked or unstacked thylakoids.

Again using the antibody method, we have shown that FNR is located far enough from PS I so that the antibody against this enzyme (the diameter of a 7-S γ globulin is approximately 15 nm) does not produce any steric hindrance to the interaction of PS I with ferredoxin (Forti et al. 1983). This is why ferredoxin has to be a freely diffusing molecule on the stroma side of the thylakoids, as plastocyanin is diffusing in the lumen, transferring electrons from the cytb6/f complex to the oxidizing side of PS I.

In the late seventies, the phosphorylation of thylakoid proteins by a thylakoid bound kinase was discovered by Bennett (1977). LHC IIb seemed initially to be the most important phosphorylated polypeptide (Bennett 1977; see a review by Allen 1992), and a general consensus was quickly reached on the

concept that phosphorylation of LHC II is the essential biochemical event involved in the state 1–state 2 transition, discovered by Bonaventura and Myers (1969). It was established that phosphorylation of a fraction of LHC IIb was the cause of its lateral diffusion in the membrane, displacing it from the PS II to the PS I antenna (see a review by Allen 1992). Dephosphorylation was involved in the reverse transition to state 1. We joined the crowd trying to understand all the implications of this regulation. The decrease of the PS II antenna size which follows the phosphorylation of LHC II was measured in isolated thylakoids. A corresponding increase of PS I antenna, and the decrease of the Emerson enhancement (see Emerson et al. 1957; R. Govindjee et al. 1962) by long wavelength light, was observed as expected (Forti and Fusi 1990; Forti and Vianelli 1988). How the phosphorylation of LHC II (and other membrane proteins) determines the migration of part of this chlorophyll-protein complex from the PS II to the PS I antenna and the associated state transition is far from being understood (Allen 1992). Nevertheless, the number of papers on this subject has been decreasing enormously in the recent years, while interest has now shifted to photoinactivation (photoinhibition) and 'protection' from it, probably also as a response of the research community to the request from the granting agencies for more applied research.

Regulation of electron transport: Rates and alternative pathways

Our interest in the regulation of photosynthetic electron transport developed at two levels: (a) the influence of protons produced within the thylakoids (or separated across the membranes, generating ΔpH) by electron transport at specific redox reactions, such as the primary photochemical reaction of PS II or the well-known rate limiting step at the level of plastoquinol oxidation; (b) the choice of the pathways and regulation of the rates of electron transport commanded by the difference of the stoichiometric ratios of ATP to NADPH respectively produced by linear electron transport and utilized by CO_2 assimilation. These two aspects of regulation are necessarily studied with different methods and approaches, but really should merge in a coherent, unitary picture.

The influence of protons on PS II turnover was initially studied (with Alex Ehrenheim and Giovanni Finazzi) in the steady state, in isolated thylakoids under conditions where PS II primary photochemistry was made rate limiting. On the basis of the effect of nigericin and of $\Delta\Psi$ suppressing ionophores, we found that membrane-localized protons, and not the membrane potential was responsible for inhibition of PS II turnover (Ehrenheim et al. 1991; Finazzi et al. 1992). Then, Giovanni started a PhD research project on this problem, and he performed the analysis of reduction of Q_A, the first plastoquinone electron acceptor of PS II, with the classic chlorophyll *a* fluorescence induction method (see Govindjee 1995 for a historical perspective). Giovanni learned how to use the models to evaluate the relevant parameters and, initially with the cooperation of R. Bianchi, the statistical methods to be utilized. We could conclude that thylakoid-localized protons do control PS II turnover, and that a few turnovers are sufficient to set up the control mechanism (Finazzi et al 1995). Thanks to the hospitality of Pierre Joliot at the Institut de Biologie Physico-Chimique in Paris, Giovanni Finazzi became familiar with the use of the methods and instrumentation constructed by Joliot and his associates over the years, and he studied the mechanisms and regulation of electron transport and the associated formation of the 'slow phase' of membrane potential at the level of the *cyt.b6/f complex* in vivo, in unicellular green algae (Finazzi and Rappaport 1998).

The second aspect of the problem of regulation, namely, whether the extra ATP needed for CO_2 reduction to the sugar level with respect to the amount of it produced by the linear electron transport to NADP is coupled to cyclic electron transport or to the ascorbate-Mehler reaction, or possibly, to either the former or the latter under different conditions, has been one of my interests for a long time, and the work on it has been resumed recently. *My old conviction that cyclic photophosphorylation was an essential part of photosynthesis (Forti and Parisi 1963) faded considerably during the years, as a large body of evidence accumulated in favour of the Mehler reaction, or rather a modification of it, as the pathway of electron transport (alternative to NADP reduction) which fulfils the role of providing ATP in the amount needed to run the Calvin cycle.* Indeed, the light dependent O_2 uptake, indicative of the Mehler reaction, has been shown in different laboratories, with differently conceived experiments. It has been reported that O_2 is constantly consumed during steady state photosynthesis in intact chloroplasts (Egneus et al. 1975). Jennings and Forti (1974b) demonstrated that O_2 is required to produce ATP in isolated intact chloroplasts to overcome the

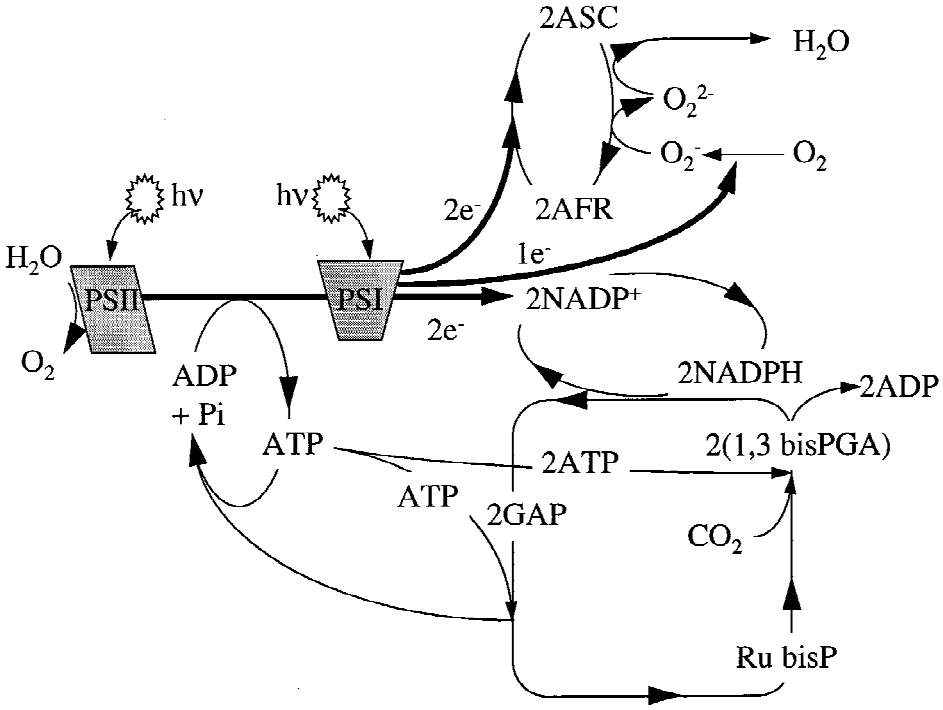

Figure 3. Scheme of the regulation of the alternative reduction of NADP and ascorbate Mehler reaction at the reducing side of PS I. See text for an explanation. PS II – Photosystem II; PS I – Photosystem I; ASC – ascorbate; AFR – ascorbate free radical; GAP – glyceraldehyde3-phosphate; Ru bisP – ribulose 1,5 bis phosphate.

lag of CO_2 assimilation, where no lag is observed and no O_2 is needed for the onset of oxaloacetic reduction, an NADPH dependent reaction which does not require ATP. Forti and Gerola (1977) have measured the initial rate of light dependent H_2O_2 formation upon addition of cyanide to isolated chloroplasts during CO_2 assimilation. This production of H_2O_2 was interpreted as due to the uptake of O_2, forming O_2^- then the peroxide, which cannot be disproportionated by the peroxidases in the presence of cyanide. All these observations are consistent with a role for O_2 uptake during photosynthesis.

After Miyake and Asada (1992) discovered that ascorbate peroxidase is present in the chloroplasts both bound to thylakoids and soluble in the stroma, we revisited the old experiments of Forti and Jagendorf (1961) on the stimulation of 'endogenous' photophosphorylation by ascorbate, and the overall picture became clear. The slow running Mehler reaction triggers the oxidation of ascorbate by ascorbate peroxidase, producing the ascorbate free radical (AFR), which is reduced by PS I at a rate which is of the same order of magnitude as that of NADP reduction (Forti and Ehrenheim 1993). ATP formation is coupled to AFR reduction with the same stoichiometry as when NADP is reduced (Forti and Elli 1995), as would be expected, because the very same electron transport system is involved (from water to the reducing end of PS I, see Figure 6 in Forti 1996). The shift from NADP to AFR as the terminal acceptor is simply determined by the fact that NADP is only present in catalytic amounts in the chloroplasts, and after it has been reduced to NADPH it can be reoxidized only if 1,3 bisPGA is available. The availability of 1,3 bisPGA obviously requires ATP and PGA. Thus, the concentration of ATP regulates, at the reducing end of PS I, the reduction of NADP or the ascorbate free radical in response to the requirements for CO_2 assimilation. Figure 3 shows a scheme of this. The sequence of events is as follows (see Forti and Elli 1995): O_2 is reduced to O_2^- at the acceptor side of PS I by the Mehler reaction (a rather slow reaction), then ascorbate is oxidized to AFR by O_2^-, a very fast spontaneous reaction which produces O_2^{2-}. The peroxide oxidizes more ascorbate to AFR through the catalysis of ascorbate peroxidase, a thylakoid bound enzyme (Miyake and Asada 1992), and finally PS I reduces rapidly AFR (Forti and Ehrenheim 1993) and the electron transport is coupled to

ATP synthesis with the same stoichiometry as in the case of NADP reduction (Forti and Elli 1995). When ATP is formed, PGA is phosphorylated to 1,3-bisPGA and NADPH can be reoxidized, so that the electrons flow again to NADP.

Another reason, though speculative, to believe that the ascorbate-Mehler reaction rather than the cyclic electron flow around PS I is operating for the synthesis of the ATP required, is that the Mehler-ascorbate system utilizes the energy absorbed by both photosystems, whereas cyclic only involves PS I; accordingly, most of the PS II absorbed energy would be lost. Indeed, even in state 2 only 15 to 20% of the PS II absorbed energy is transferred to PS I in higher plants (see Allen 1992). The situation is different in unicellular green algae. In these micro-organisms it has been shown that approximately 80% of LHC II is incorporated into the antenna of PS I during the transition to state 2, its size increasing by approximately 50% (Delosme et al. 1996). Therefore, it could be that in higher plants the state transitions are a mechanism for balancing the two photosystem activities, while in the microalgae PS I activity and cyclic photophosphorylation would prevail in state 2. The future will tell whether such a hypothesis has any merit.

Acknowledgments

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