The birth of the photosynthetic reaction center: the story of Lou Duysens

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The birth of the photosynthetic reaction center: the story of Lou Duysens

Rienk van Grondelle · Hans van Gorkom

The reaction center concept

The concept of photosynthetic reaction centers originated as one of the proposed interpretations of the legendary experiments by Emerson and Arnold 1932 (Myers 1994), which showed that the illumination of *Chlorella* cells by short saturating flashes reduced only one CO_2 molecule per 2,500 chlorophyll. Gaffron and Wohl (1936; Wohl 1937) suggested that the excited state might be transferred over many chlorophylls until it is finally trapped at a special place where the chemical reaction is initiated, and that the number of such reaction centers limits the flash yield of CO_2 assimilation. They were right, but at that time it was not yet possible to verify such speculations. Moreover, Franck and Livingston (1949) "proved" that the transfer of electronic excitations could occur over maximally 100 chlorophylls, and for that reason other interpretations of the Emerson and Arnold experiments, attributing photochemical activity to all chlorophylls, remained popular.

It was the Doctoral thesis of Louis N.M. Duysens in 1952 that established the basics of photosynthetic "light-harvesting" and the existence of reaction centers that function as efficient traps for the electronic excitation. Duysens happened to be in the right place at the right moment, and soon proved to be very much the right man at that place and that moment. According to his own account (Duysens 1989a, b), after studying physics and mathematics at the university of Utrecht, he was offered a position as a research assistant at the Biophysical Research Group, if he would do a PhD research project on Planck's constant. He insisted on working full time on a subject in photosynthesis, however, and became the first physicist in that group to do so. In the choice of subject, he wisely ignored the proposal by Wassink to study fluorescence induction kinetics and instead chose excitation transfer, being the most physical topic, the least likely to get lost in biological complexies. Clearly, Duysens was not taking advice from his superiors and followed his own line of thought from the start.

Duysens was very much inspired by the first theoretical formulation of the process of electronic excitation transfer by Förster (1947, 1948). Förster showed that two pigment molecules at sufficiently short distance and with the right orientation could exchange an electronic excitation at a rate that depended on four parameters: the inverse 6th power of the donor acceptor distance R, R^{-6}; the radiative rate of the energy donor k_R^D; a factor determined by the relative orientation of donor and acceptor κ^2 en the overlap between the donor emission $F_D(\tilde{v})$; and acceptor absorption $\varepsilon_A(\tilde{v})$:

$$W_{DA} = 8.8 \cdot 10^{17} \cdot \frac{k_R^D}{n^4} \cdot \frac{\kappa^2}{R^6} \int \frac{\varepsilon_A(\tilde{v}) F_D(\tilde{v})}{\tilde{v}^4} d\tilde{v} \qquad (1)$$

In Eq. 1 $\tilde{v}$ is the frequency in cm^{-1}.

The power of the Förster equation is that it contains mainly the experimentally accessible parameters. Absorption and emission spectra were measured and the distance between neighboring chlorophylls was estimated from the chlorophyll concentration. The orientation parameter was typically taken as $\kappa^2 = 2/3$, the average value for a random distribution of donors and acceptors. Note that, the equation for the rate of energy transfer as given by Förster holds for the truly incoherent case, in which the excitation is

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perfectly localized on a single pigment and performs a random walk ("hops") on a lattice of chlorophyll molecules until a trap is reached and is converted into a product involving oxidizing and reducing species.

The effective number of such hops is related to the fluorescence quantum yield of the system. Assuming a fluorescence yield of 0.1 % led Franck and Livingston to their estimate of <100 steps, which they considered as insufficient for the reaction center concept to work. However, Duysens pointed out that published values of the fluorescence yield required several corrections leading to a values around 1 %. In addition, he proposed that the limiting reaction in the experiments of Emerson and Arnold was more likely a one quantum process in reaction centers present at a concentration of 1 per 200 chlorophyll, rather than the 10 quantum process of CO_2 reduction in a unit present at ten times lower concentration. Together, these considerations made the "reaction center" hypothesis a realistic possibility.

Reaction centers in purple photosynthetic bacteria

In earlier experimental study using fluorescence excitation spectroscopy, Duysens had firmly established that in photosynthesis energy transfer that occurs between high- and low-energy pigments in a variety of photosynthetic organisms: energy transfer from carotenoids to bacteriochlorophyll in purple bacteria, from carotenoids and chlorophyll *b* to chlorophyll *a* in green algae, and from phycobilins to chlorophyll *a* in cyanobacteria (Duysens 1951, 1952). On this basis, he was fully convinced of the "energy transfer-reaction center" hypothesis, and he set out to prove this idea. Assuming that there was a reaction center, then, following Förster, its absorption had to overlap with the emission of the long-wavelength (bacterio-)chlorophyll. He further hypothesized that in case the reaction center pigment, which he named P, underwent some photochemical conversion, its absorption would disappear. Duysens chose to use the purple bacterium *Rhodospirillum rubrum* since Wassink et al. (1942) had demonstrated that its fluorescence increased strongly upon prolonged illumination, consistent with the spectroscopic removal of the energy trapping pigment P. For *Rhodospirillum rubrum,* Duysens estimated from the observed fluorescence quantum yield that P would be present in a 1:100 ratio, based on Förster's theory.

Using the parts of a fluorescence spectrophotometer, Duysens constructed an absorption difference spectrophotometer that allowed him to observe light-induced absorption changes of this size. It turned out to be relatively easy to observe a reversible bleaching at 890 nm indicating that about 3 % of the bacteriochlorophyll molecules got bleached at saturating light intensities. Figure 1, a copy of the original figure in his PhD thesis, shows the reversible absorption changes in cells of the purple bacterium *R. rubrum* and in an "aqueous extract" of *Chromatium vinosum* induced by illumination.

Duysens concluded from these observations that a small fraction of the bacteriochlorophylls functions as a reaction center where the electronic excitation is converted into a photochemical product. Based on the effect of oxygen and depletion of the electron donor, it was suggested that P got oxidized, while another molecule got reduced (Duysens et al. 1956, reprinted and commented on in Duysens 1989a). The idea of a reaction center that traps the excitation from the surrounding bacteriochlorophylls to drive a charge separation was born and today the reaction center concept is one of the cornerstones of our understanding of photosynthesis.

One anomaly in the original experiments by Duysens that apart from the bleaching of P, a bandshift signal around 800 nm could be observed, could later be explained by the electrostatic effect of the positive charge on P on the absorption properties of a nearby pigment in the reaction center absorbing around 800 nm. The structure of the bacterial reaction center resolved by Deisenhofer et al. (1984), beautifully demonstrated the position of the B800 bacteriochlorophyll nearby P, then identified as the (special pair) of bacteriochlorophylls located in the heart of the reaction center.

Later, similar light-induced absorption difference spectra were observed in other photosynthetic bacteria and algae, further supporting the concept of the reaction center. Applying absorption difference spectroscopy in different spectral regions, Duysens observed a multitude of other signals related to the primary processes of photosynthesis such as the oxidation of a cytochrome in the purple bacterium *R. rubrum* (Duysens 1954a), the "515 nm signal" in *Chlorella* (Duysens 1954b) and the oxidation–reduction of a cytochrome in *Porphyridium cruentum* (Duysens 1955). The latter was the prelude to the discovery of the two photosystems.

Two photosystems operating in series

Indications for the presence of two photosystems came from chlorophyll fluorescence experiments on cyanobacteria, where it was shown that fluorescence excited by the phycobilins had a much larger quantum yield than fluorescence measured via direct chlorophyll excitation. The hypothesis was then that two photosystems existed, one excited selectively via phycobilins and highly fluorescent, the other only excited via chlorophyll and weakly fluorescent (Duysens 1951, 1952; see also Govindjee and Rabinowitch 1960, Butler 1962).

About a decade later, using absorption difference spectroscopy and varying the excitation wavelength between

Fig. 1 The reversible light-induced bleaching of a small fraction of bacteriochlorophyll for the first time observed by Lou Duysens and reported in his thesis
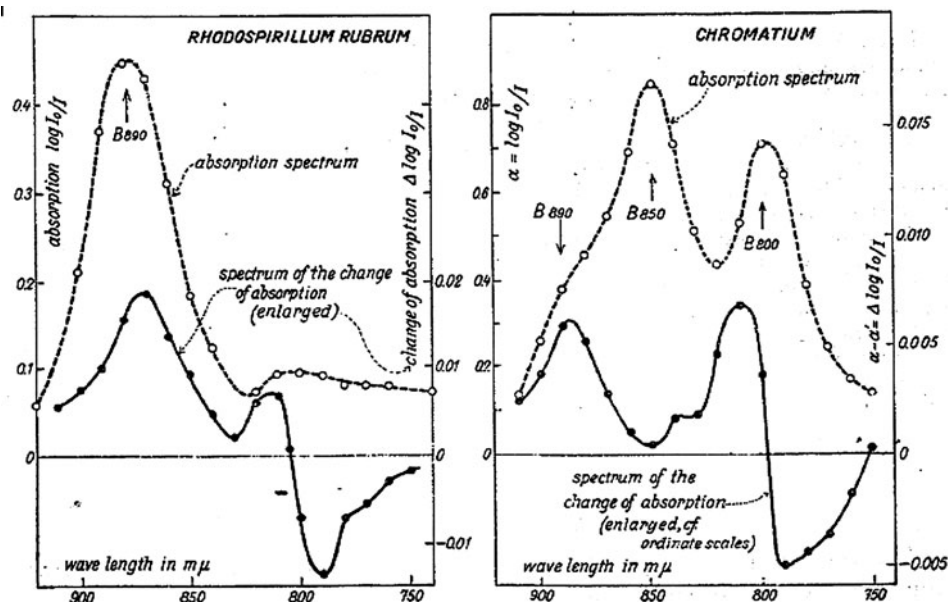
Fig. 8.3. Absorption spectrum and spectrum of the reversible change of absorption at irradiation of *Rhodospirillum rubrum* in tap water. The ordinate of the latter spectrum is plotted on a scale ten times smaller than that of the former. A decrease in absorption on irradiation is plotted as a positive value of the ordinate, an increase as a negative one. The location of the main absorption maximum of the bacteriochlorophyll type *B* 890 is indicated by "*B* 890".

Fig. 8.4. Absorption spectrum and spectrum of the reversible change of absorption at irradiation for a colloidal aqueous extract of *Chromatium*. The ordinate of the latter spectrum is plotted on a scale fifty times smaller than that of the former. Positive values of the ordinate indicate a decrease, negative ones an increase in absorption. The location of the absorption peak of each of the three bacteriochlorophyll types (see Introduction) is indicated in the figure by "*B* 890", "*B* 850" and "*B* 800", respectively.

phycobilin excitation (mainly exciting the fluorescent pool of chlorophylls) or far red excitation >690 nm (mainly exciting the weakly fluorescent pool of chlorophylls) and measuring cytochrome oxidation and reduction, it was observed that the latter (Light 1) oxidized the cytochrome, while the former (Light 2) reduced the cytochrome. Based on these experiments, it was correctly concluded that the two proposed photosystems operate in series (Duysens et al. 1961).

Building on these experiments, measuring the rate of phosphopyridine nucleotide (NADP) reduction by fluorescence and cytochrome oxidation by difference absorption, it was concluded that both action spectra contained contributions from phycocyanin and chlorophyll *a*, but a relatively higher contribution from chlorophyll *a* than the action spectrum for photosynthesis. The results, together with other evidence, support the hypothesis that in algal photosynthesis two different photochemical oxidation–reduction reactions occur, mediated by two different pigment systems. The two systems operate in series: the first one, Photosystem 1, reduces phosphopyridine nucleotide and oxidizes the cytochrome, and the other one, Photosystem 2, reduces the cytochrome and produces O_2 by

means of the oxidation of water (Duysens et al. 1961; Duysens and Amesz 1962). These experiments provided the experimental verification of the famous Z-scheme of photosynthesis, originally proposed by Hill and Bendall (1960) and now a prominent textbook feature.

A further essential observation was that the fluorescence yield of all oxygenic photosynthetic systems depends on the redox state of a quencher called Q (Duysens and Sweers 1963), hoping that it would prove to be a quinone… It was observed that light 2 reduced the quencher in a one quantum process, as manifested by a fast rise of the fluorescence, while light 1 oxidized the quencher and resulted in a decay of the fluorescence to its original "dark" level. When biochemical, structural and spectroscopic information became available, Q turned out to be the strongly bound quinone Q_A in all type 2 photosynthetic reaction centers.

The photosynthetic reaction center stores solar energy

Reaction centers not only trap electronic excitations but also convert the solar energy into useful chemical work.

Duysens was the first to realize that the second law of thermodynamics imposes a limit on the efficiency of putting sunlight to work (Duysens 1958). He argued that the lowest light intensity at which maximal photosynthetic efficiency is reached corresponds to that of black body radiation at about 1,100 K, while the temperature of the storage is 300 K. According to the second law of thermodynamics, this yields a maximum efficiency of 73 % for algae/plants. This is more readily seen in terms of kinetics (Ross and Calvin 1967)—photosynthesis would run in reverse unless the products are formed faster than they are lost that way. Their stabilization relative to the natural lifetime of the excited state, required to make their loss rate smaller than the excitation rate, implies a standard free energy decrease corresponding to the limitation by the second law (Knox 1969). Now that, this is no longer disputed. Duysens is rarely cited for it, but it is not trivial, and at the time, it was not readily accepted.

Excitation energy transfer

As discussed above, the discovery of efficient excitation energy transfer in photosynthesis was at the basis of the reaction center concept. In later study, Lou Duysens developed the so-called matrix model for photosynthetic excitation energy transfer, or also called the Vredenberg–Duysens model (Vredenberg and Duysens 1963; Duysens 1978). The observation was that there exists a linear relation between the reciprocal of the fluorescence yield and the fraction of closed (or inactive) reaction centers. The simplest model that explains this phenomenon is a large antenna (much larger than the diffusion length of an excitation) coupled to many reaction centers. In case one reaction center is closed, there is a large probability that the excitation can visit another neighboring active reaction center and still be trapped. In the limit of ultrafast energy transfer the Vredenberg–Duysens relation can be reproduced (Duysens 1978), although that is not an absolute requirement. Experiments in which the exciton diffusion length was measured using either singlet–triplet (Kolubayev et al. 1985) or singlet–singlet annihilation (Bakker et al. 1985) demonstrated the extensive mobility of photosynthetic excitons over many hundreds of chlorophylls and provided strong support for Duysens' matrix model.

How it all came together

During the final years of his professorship in Leiden, Lou Duysens was blessed with a working picosecond transient absorption spectrophotometer and some excellent collaborators, including Cees van Bochove, Tom Nuijs, and especially Vlad Shuvalov. We are happy to have been part of

that team. It must have been a great satisfaction for Lou to see all his concepts and predictions become reality in the beautiful set of results that this team collected on membrane and reaction center preparations of purple photosynthetic bacteria (Nuijs et al. 1985b; Shuvalov and Duysens 1986; Shuvalov et al. 1986b), green photosynthetic bacteria (van Bochove et al. 1984; Nuijs et al. 1985c, 1986c; Shuvalov et al. 1986a, d), photosystem 1 (Nuijs et al. 1986b; Shuvalov et al. 1986c), photosystem 2 (Nuijs et al. 1986a) and heliobacteria (Nuijs et al. 1985a).

The experimental study on photosynthetic energy transfer and charge separation has made amazing progress during the quarter century following the retirement of Lou Duysens. However, we would never be where we are now without his insights and experimental skills. During the mid-90s, Lou found a few copies of his thesis in his house and he asked who might be interested. One copy was sent to Graham Fleming, one of the major energy transfer players during the last 25 years. After receiving it he answered: "Sometimes I wonder why we are all doing this. It has been done!"

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