

# Membrane Model to Explain the Participation of Carotens and the Changes in Energy in Photosynthesis

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**Abstract.** We have found several bands in the visible and near infrared that are related to the primary process of photosynthesis. Starting from

the value of 1830 mV (677.5 nm) which was found by some authors and corresponds to activated PSII, there is a first loss of 580 mV leading to a carotenoid cation (Phe/Car<sup>+</sup>) formation which is in close contact with pheophytin (Phe) in accordance with previous works leaving P680/P680<sup>+</sup> with an energy of 1250 mV. We propose that in this process the carotenes may change their stereochemistry from *trans* to *cis* thus avoiding the electron return path.

**Key words:** Photosynthesis, Tyrosine Z, Carotenoids, P680, Pheophytin.

**Resumen.** Hemos encontrado varias señales en el espectro de visible e infrarrojo cercano que están relacionadas con el proceso primario de la fotosíntesis. Empezando con el valor de 1830 mV (677.5 nm) que ha sido reportado por otros autores, a partir del cual hay una pérdida inicial de 580 mV y llegando a una señal del catión carotenoides y la feofitina (Phe/Car<sup>+</sup>) que se encuentra en contacto próximo con feofitina (Phe) de acuerdo con trabajos anteriores, dejando el par redox P680/P680<sup>+</sup> con una energía de 1250 mV. Consideramos que en este proceso los carotenos sufren un cambio en su esteoquímica de *trans* a *cis* impidiendo así el regreso de los electrones por esa vía.

**Palabras clave:** Fotosíntesis, Tirosina Z, Carotenoides, P680, Feofitina.

## Introduction

Several recent papers have appeared which deal with the participation of the carotene cation in photosynthesis [1,2,3,4,5]; these papers have shown the presence of a carotene with a positive charge and the effect in the spectra of PSII photocenter. However most of these studies have been carried at very low temperatures (273 °K) which are not compatible with a working photocenter since the optimal temperature for photosynthesis for a large number of plants is between 283 °K and 293 °K [6]. An exception to these limits is found in some thermo stable photosynthetic bacteria.

In recent research, X ray structural information has been incorporated into the energetic scheme and it has been possible to relate spectroscopic data to this complex scheme. An example is found in the review by Dinner and Rappaport [7]. In a paper by Noguchi *et al.* [2] they studied the electronic absorption spectra of PSII RC (Photosynthetic System two Reaction Center) complexes and its light induced difference spectra. They found carotene and chlorophyll absorptions before and after irradiation at 150 °K. A peak appear in the near infrared region at 1004 nm and is notable as a result of fluorescence, it is assigned to a Carotenoid with a positive charge that is Car<sup>+</sup>. There are several more recent reports on the presence of a Car<sup>+</sup> but they place the immediate absorbance at 990 nm and this band is shifted to 1008.8 or 1009 nm with time. Actually, the band shifts under oxidation or reducing conditions and may be

detected also by EPR in the presence of 5 mM K<sub>3</sub>[Fe(CN)<sub>6</sub>] as oxidant or 3 mM ascorbate as reductant [3]. In structural studies five different X ray structures of PSII, have been reported [8, 9, 10, 11, 12]. Unfortunately all this five studies have been carried in bacteria and there is no similar study in plants.

It is well known that plants and photosynthetic bacteria share a similar biochemical machinery to carry out photosynthesis [14]. However, the plant system has more antenna complexes [14]. It is important to remember that all these processes are carried out in a membrane system and these should be also taken into consideration. In bacteria there are two proximal antenna complexes in some of the X ray crystals systems studied [12]. In these studies two very close chlorophyll molecules appear in the PS II center which may interact through their  $\pi$  electron system, these molecules are labeled as P<sub>D1</sub> and P<sub>D2</sub>, there are also two additional chlorophyll molecules, labeled as chlorophylls D1 and D2 at a short distance [12].

In plants this system is capable to send one electron to a close pheophytin molecule labeled as Pheo which we are going to investigate in plants [15].

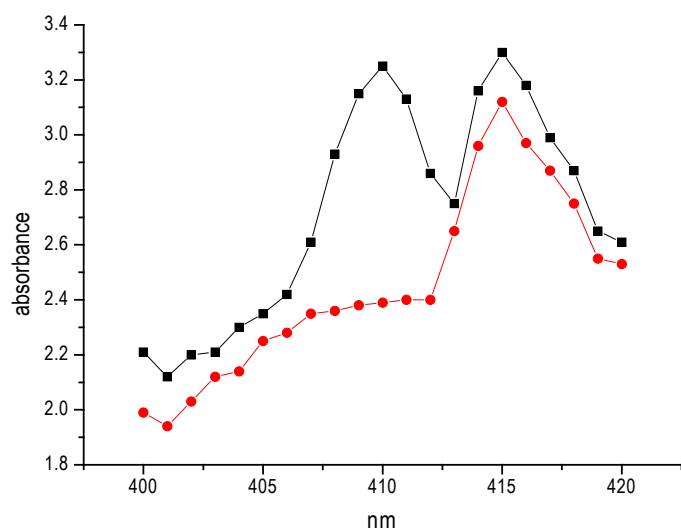
## Results and Discussion

We consider that if there is postulate that exist a difference in redox potential between Z/Z' (Z: electron donor; Tyrosine) and D/D' radicals, this should be reflected mainly in the higher

occupied molecular orbital (HOMO) of between them. For this reason we decided to compare in more detail the spectra of these radicals in the region between 400 and 425 nm. The spectra reported by  $Z^{\bullet}$  [1,19] show a peak at 410 nm that correspond to the HOMO orbital of  $Z^{\bullet}$ . Our experimental results are somewhat similar to those reported earlier, however, our interpretation is different. We were able to observe found a peak at 410 nm immediately after irradiation but after few seconds of irradiation only the peak at 415 nm remains, this is assigned to the HOMO orbital of  $D^{\bullet}$  (Figure 1), it is necessary to subtract the background due to Quinones  $Q_A$  and  $Q_B$ , which also absorb in this region as has been done before [18].

The experimental value of the redox potential for tyrosine  $D/D^{\bullet}$  is 760 mV at pH 8.5 as described by Boussac and Etienne [20]. This value must be corrected for the actual pH of D which is located in the lower end of the membrane and thus has an acidic pH which has been estimated by for this model as 4.74 in accordance with our membrane model (figure 3). We have to add a correction by the pH effect which amounts to 224.4 mV. In order to study the pH effect in detail we have used as a model the recently published structures of PSII [8,9,10,11,12], see (figure 2).

In this model the pH of 6.1 corresponds to the middle part of the membrane since there is a difference in pH of 2.72 as derived from the value of 161 mV, which we call "the membrane potential". This value has been mentioned earlier by Heinze [17]. Then the top part of the membrane should be at a pH of 7.46 (relatively basic) and corresponds to the top line traced between the iron atom and the bicarbonate ion. In the low part of this membrane the pH should be 4.74 (relatively acidic) at the line between Z and the four manganese atoms. At this pH the corrected value for the potential for  $D/D^{\bullet}$  derived from the Nernst equation at 25°C should be  $986.4 \pm 20$  mV. Then



**Figure 1.** Highest occupied (HOMO) molecular orbitals for  $Z^{\bullet}$  and  $D^{\bullet}$  radicals. The highest points shown (squares) means the HOMO spectra observed during Irradiation for  $Z^{\bullet}$  and  $D^{\bullet}$  and points shown as diamonds correspond to the observation after 20  $\mu$  sec.

the difference in energy which we obtain between the HOMO orbital's of  $Z/Z^{\bullet}$  and  $D/D^{\bullet}$  is 36.4 mV which we calculated from the spectroscopic wavelength  $\lambda_{\max}$  difference, using the well known equation:

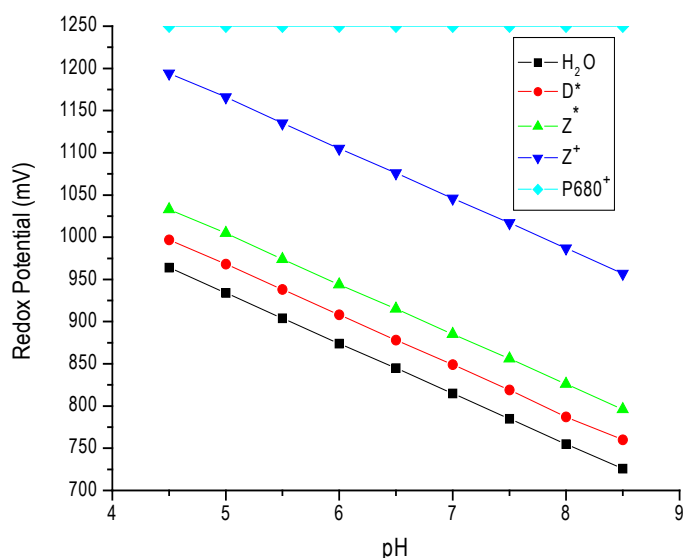
$$E = hc/\lambda_{\max}$$

The value of  $986 \pm 20$  mV plus the difference between  $Z/Z^{\bullet}$  and  $D/D^{\bullet}$  of 36.4 mV finally gives a value of  $1021 \pm 25$  mV for  $Z/Z^{\bullet}$ . This energy difference of 36.4 mV is considerably lower than the value mentioned earlier [2].

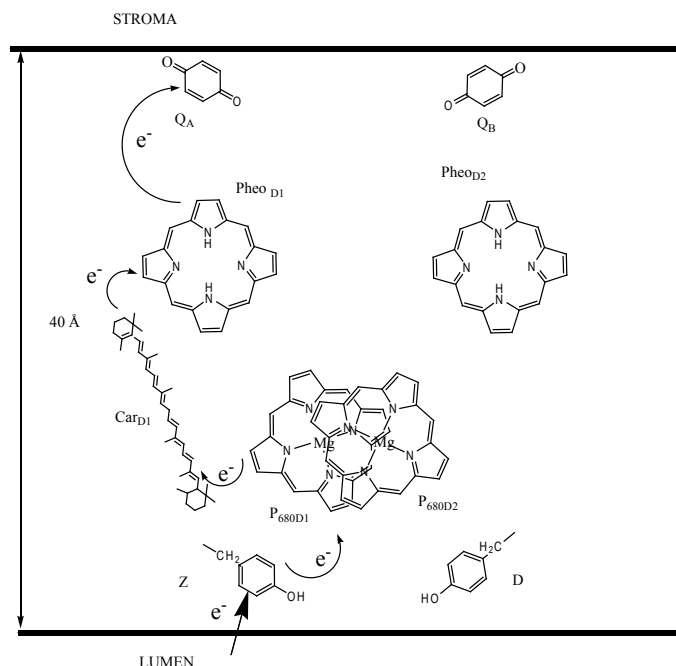
A somewhat smaller value of 32 mV is found by using the difference between the maxima at 440 nm due to an electrochromic effect of Z and the corresponding at 445 nm due to the electrochromic effect of D tyrosine, also on a chlorophyll molecule. However, it must be noted that the band at 440 nm has been attributed to a chlorophyll molecule and not to Z according to Diner *et al* [1]. So this leaves the maximal at 410 and 415 nm as the only logical candidates for the HOMO of  $Z^{\bullet}$  and  $D^{\bullet}$ , respectively. The afore mentioned radicals include an electron which is precisely in the HOMO, so the difference in energy should correspond to a difference in oxidation potential of this radicals although there could be also a difference due to a pH effect.

The experimental pH of 6.1 that we used is optimal for oxygen evolution [17, 21] in spinach leaves, and this has considerable implications since it settles the working pH of the participating components in the membrane system, not all components are at the same pH, thus the experimental pH of 6.1 it settles the working pH.

For our working model membrane, there should be a gradient of potential from the stroma to the lumen of 2.72 pH units which is based on a difference of 161 mV. Our results are in



**Figure 2.** A Membrane Model for PSII Photocenter. Only the position of the main redox components is shown. This model is in accordance with the x ray information available. Redox potentials of P680<sup>+</sup>, Z<sup>+</sup>, Z<sup>•</sup>, D<sup>•</sup> and H<sub>2</sub>O, at different pH values.



**Figure 3.** Potential variation as a function of pH for water, tyrosine’s Z° and D°, Z+ and water between pH 8.5 and 4.5 together with the maximum available potential of Car/Pheo+. This figure shows and scheme of our membrane model of photosynthetic apparatus, showing the main components in a partial electron path during the photosynthesis processes.

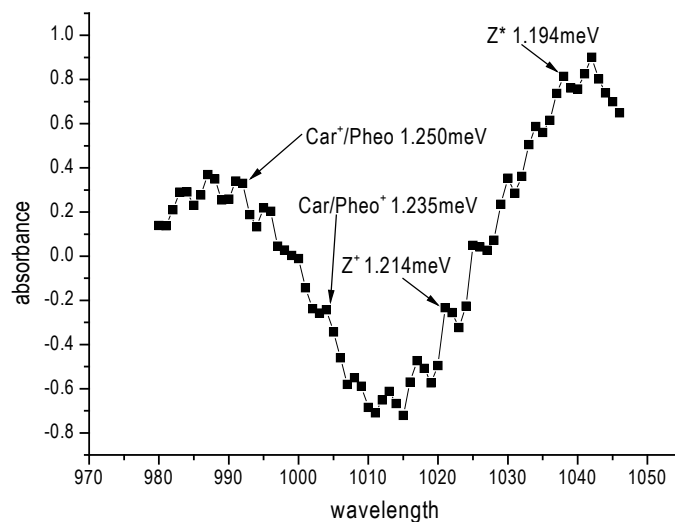
accordance with recent findings of Grabolle and Dau [22]. They have published results for the redox potential of P680/P680+ value of 1.25 V and for Z/Z+ of 1.21 V. This last result is larger than our value which corresponds to Z/Z\* (from Z/Z+ → Z/Z\* + H+) by about 161 mv. This difference is interpreted here as the membrane potential and is related to a Δ pH difference of 2.72. We suggest to call membrane potential for the value of 161 mV. may now be generally called the membrane potential. A similar value has been mentioned by Gibasiewicz et al [23] for the upper Quinone part of the membrane.

The roll of two carotenes in the core of PSII should be discussed further. In the most recent X ray study of PSII at 3 Å resolution by Loll et al. [8], two carotene molecules were found placed near the core PSII chlorophylls. However, their role is only partially explained, the carotene on the left side seems to play an electron conducting roll according to Peter Faller et al [4]. However, the role of the carotene molecule on the right has not been clearly explained. The spectral data from Noguchi [2] seems to indicate that one or both of the carotenes participate in electron transport. We have found spectral evidence in agreement with this proposal absorption at 992 nm in, which has been observed by us, in the region between 980 and 1050 nm (figure second derivative). This corresponds to energy of 1.249 mV; which may be related to the energy of +1.25 mV for P680/P680+ as found by Grabolle and Dau [22]. In this last paper appears also. The redox potential of Z/Z+ as +1.21 mV; this last redox potential correspond with a tyrosine

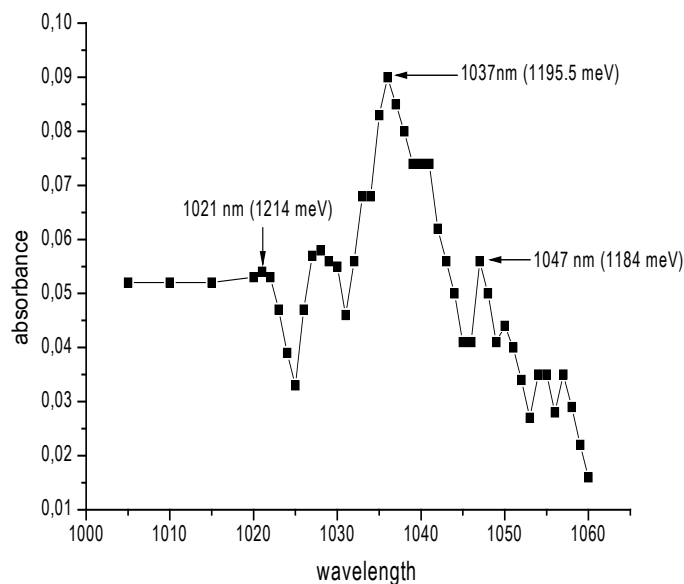
residue still holding a positive charge due to the loose of one electron; however this radical liberates a proton, which represents in the first stage an energy lose of 35 mV according to Jeans et al (24) or 38 mV according to Grabolle and Dau [22]. The second stage represent a further lose of 20 mV to give a free radical. The energy for the transport of the proton across the membrane means a further loses of 161 mV in accordance with Gibasiewicz et al [23]. This scheme brings concordance between the data of Grabolle and Dau [22] and that of Noguchi et al [2]. We have also found near infrared bands at 1020.4 nm and 1037 nm which confirm the values mentioned above. The first one corresponds to 1215 mV and the last to the value of 1195 mV in accordance with the values calculated by Grabolle and Dau [22] as shown in figures 4 and 5.

### Conclusion

Starting with energy of 1830 mV as mentioned by Grabolle and Dau [22] for P<sub>D1</sub>; electrons are send travels trough the pair Car+/Pheo to Car/Pheo+ as shown in figure 2. Electrons are then drawn from Z to yield the pair Z+/Z (figure 2.), which may loss a proton to yield Z\* as a neutral radical. In this processes a proton is lost with an additional energy loss of 161 mV, in accordance to our membrane model and the results found in this work all agree with the value mentioned by Grabolle and Dau [22]; Dau and Sauer [25] and Gibasiewicz [23]. The use of this membrane model may be extended to other fields of biological interest, for example it could be applied to the bioenergetics of ATP synthesis as well as to photorespiration.



**Figure 4.** Second derivative of the near infrared spectra of PSII together with the position of some important peaks in the region between 980 and 1050 nm as a result of irradiation at a 90° angle with a Xenon lamp. The arrows point some energy values in milielectron volts (mV). Second derivative of near infrared spectra of PSII particles. The arrows show small peaks due to the electronic pairs with different charge distribution and the corresponding energy in mV.



**Figure 5.** Partial fluorescence spectra of  $Z^+$  in the near infrared spectra as a result of irradiation of PSII particles at a  $90^\circ$  angle with a Xenon lamp in the region between 1005 and 1100 nm. The arrows point at some important peaks. Near infrared spectra in the region between 1000 and 1060 mV which is central to the energies shown by the arrows.

## Methods

Highly active PSII membranes were prepared from spinach chloroplasts as described by Van Leeuwen [16] and stored at  $-60^\circ\text{C}$  at a concentration of 2.5 mg Chl/mL in a buffer containing 50 mM Mes pH 6.1 (this pH is optimal for photocenter stability) [17]; 0.4 M sucrose, as well as 10 mM NaCl, 10 mM  $\text{CaCl}_2$ , 5 mM  $\text{MgCl}_2$  and 600  $\mu\text{M}$  potassium ferricyanide as an electron acceptor, 0.4 % dodecyl maltoside and 0.4 % of Ficcol. The spectra were observed following Haumann's procedure [18] and the spectroscopic observations were carried in a modified SHIMATSU spectrophotometer (model UV160U) in order to be able to irradiate the samples at a  $90^\circ$  angle. Irradiation was carried out with a Xenon lamp (100 watts) under continuous irradiation and recorded at each nanometer; the data shown represent the mean of five independent readings. The readings were subtracted from those of non-irradiated samples of the same composition. The presence of tyrosine  $Z^+$  and  $D^+$  radicals under irradiation was confirmed by electronic paramagnetic

resonance using a Jeol JES-TE 300 instrument at liquid nitrogen temperature.

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