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Light-induced Fourier transform infrared spectrum of the cation radical $P680^+$

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Abstract

The structure of the primary electron donor of photosystem II, P680, is still under debate. It is not decided if it is composed of a chlorophyll (Chl) monomer or dimer. In this study, Fourier transform infrared (FTIR) spectroscopy was used to analyze the changes in the vibration modes occurring upon photooxidation of P680 in a Mn-depleted PS II preparation. It is demonstrated that illumination of the above in the presence of artificial electron acceptors results in a light-minus-dark absorbance change typical of the formation of $P680^+$. The light-minus-dark difference FTIR spectrum obtained under similar conditions is characterized by two negative peaks located at 1694 and 1652 or 1626 cm^{-1} that can be assigned to the 9-keto groups of the P680 Chl, the latter band being indicative of a strongly associated group. These vibrations are shifted to 1714 and 1676 cm^{-1}, respectively, in the positive features of the difference spectrum attributed to $P680^+$. The occurrence of two pairs of bands attributed to 9-keto groups is discussed in terms of P680 being formed of a Chl dimer.

Key words: Photosynthesis; Photosystem II; P680; Chlorophyll; Fourier transform infrared spectroscopy

1. Introduction

Photosystem II (PSII) of higher plants catalyses the photooxidation of water. The electrons produced are used to reduce a pool of plastoquinone on the acceptor side of PSII. All the intermediates implicated in the electron transport from water to plastoquinone are thought to be comprised in a reaction center core complex constituted of two integral membrane proteins: D1 and D2 [1]. Thee extrinsic polypeptides with apparent molecular weight of 18, 24 and 33 kDa are involved in stabilization of the Mn cluster responsible for the oxygen evolving activity of PSII and in maintaining the proper affinity for calcium and chloride ions which are required as cofactors for oxygen evolution [2,3]. The electron transport activity is initiated by charge separation in the reaction center with the formation of $P680^+$ and the reduction of a bound pheophytin. The electrons are then transferred to Q_A and Q_B, the secondary quinone acceptors [1,4]. The radical $P680^+$ is reduced by Z, that is believed to be tyrosine-161 of the D1 polypeptide [5], which in turn oxidizes the Mn cluster [6,7].

The composition of the primary Chl donor, P680, is still under debate. The homology found between the L and M subunits of the bacterial reaction center with the D1 and D2 polypeptides of PS II suggests a dimeric structure of P680 [8,9]. Following that direction, recent CD and ESR data were interpreted in favour of a dimeric P680 [10–13]. However, a detailed analysis of van der Vos et al. [14] together with absorbance-detected magnetic resonance and low temperature photochemistry studies [14–16] indicated that P680 is rather a monomer.

Difference IR spectroscopy was used at several occasions to obtain structural information on the electron transfer intermediates in higher plant PSs. In PS I, the primary donor $P700^+$ was characterized [17], whereas in PS II, IR absorption bands were suggested to arise from the changes occurring during the S_1-to-S_2 transition of the Mn cluster [18], from the radical form of the secondary donor tyrosine [19], from pheophytin and quinone acceptors [17,20–21], and from cytochrome b_{559} [22]. In this report, FTIR is used to study the light-induced formation of $P680^+$ in a Mn-depleted PS II-enriched preparation kept under oxidizing conditions. The above system was previously shown to allow the selective accumulation of Z^+ and Chl radicals [23,24]. It was shown that the Chl radical formed is $P680^+$ [25]. The position of IR absorption bands attributed to the P680 Chl can provide new information on the structure of the reaction center.

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Abbreviations: PSII, photosystem II; Chl, chlorophyll; IR, infrared; FTIR, Fourier transform infrared; P680, primary electron donor of photosystem II; Q_A, primary quinone acceptor; Q_B, secondary quinone acceptor.

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2. Materials and methods

Chloroplast particles enriched in PS II were isolated from spinach leaves by means of centrifugation at 20.000 × *g* of a chloroplast suspension treated with 0.4% digitonin and 0.15% Triton X-100 as previously described [26]. These particles (DT-20) contained 80–100 Chl molecules per molecule of photoreducible pheophytin measured spectroscopically as described elsewhere [25] and evolved oxygen at a rate of 300–350 μmol/mg Chl · h under saturating light in the presence of 0.3 mM potassium ferricyanide and 0.2 mM phenylene-*p*-benzoquinone. The complete Mn extraction (0.02 Mn atoms per reaction center left) from the DT-20 particles was achieved as in [27].

Photoinduced absorbance changes were monitored at 20°C as described previously using PS II preparations at a Chl concentration of 50 μg/ml [25]. Samples for FTIR measurements were prepared by drying the equivalent of 50 μg Chl on BaF_2 windows for 20–30 min under a light stream of dry nitrogen. The samples contained 500 μM potassium ferricyanide and 10 μm silicomolybdate added before drying. The IR spectra were recorded on a Bomem DA3-0.02 instrument equipped with a nitrogen-cooled HgCdTe detector and a KBr beam splitter. The spectra were averaged from 100 scans with a precision of 2 to 4 cm^{-1}. The spectra were recorded at 20°C before and during illumination (100 $W \cdot m^{-2}$) by a fiber optic guide connected to an Oriel model 77501 Fiber Optic Illuminator equipped with a heat filter and a red filter (> 600 nm). The difference light-minus-dark spectra were calculated using the C-H stretching bands around 2900 cm^{-1} as internal standard. The precision of the method of subtraction was tested using three different Mn-depleted samples before and after illumination under the same experimental conditions. The difference spectra obtained showed a flat baseline for the C-H stretching vibrations around 2900 cm^{-1}.

3. Results and discussion

The kinetics of absorbance changes at 678 nm are presented in Fig. 1. When PS II particles are used before removal of the manganese cluster, even under oxidizing conditions (presence of 500 μM potassium ferricyanide and 10 μM silicomolybdate) only a weak absorbance change is seen upon illumination (Trace 1). Under these

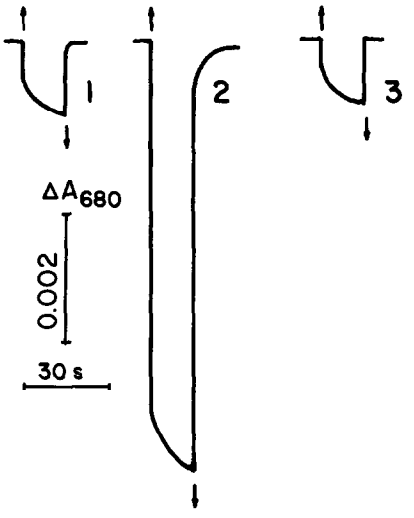

Fig. 1. Kinetics of photoinduced absorbance changes (*ΔA*) at 678 nm related to P680 photooxidation in the presence of 500 μM potassium ferricyanide and 10 μM silicomolybdate in the PS II preparations before (1) and after (2,3) a complete removal of Mn without (2) and with addition of 10 μM $MnCl_2$ (3). Up and down arrows indicate light on and off, respectively.

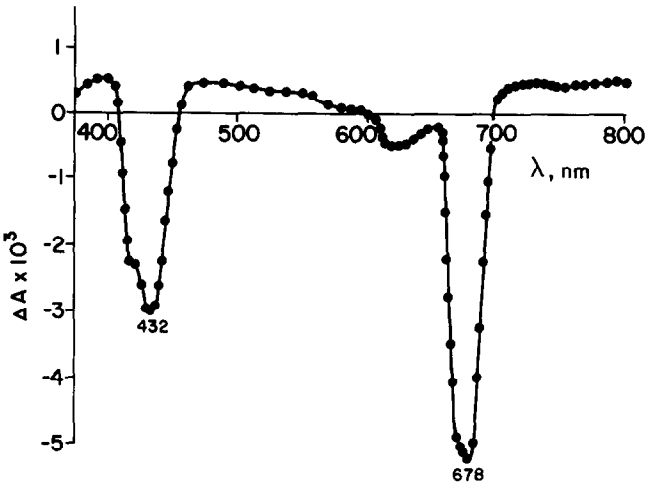

Fig. 2. Light-minus-dark difference spectrum of the reversible absorption changes seen in Mn-depleted photosystem II preparations in the presence of 500 μM potassium ferricyanide and 10 μM silicomolybdate.

conditions, the active oxygen evolving complex keeps the reaction center and the secondary donor Z mostly in the reduced form. However, after removal of the manganese cluster, inactivating the water splitting enzyme, the illumination results in a strong absorbance change reversible in the dark (Trace 2). Weak absorbance changes can be regenerated by the addition of 10 μM $MnCl_2$ to reconstitute the manganese cluster (Trace 3). These changes have been shown to originate from the formation of $P680^+$, the photooxidation product of P680, favoured when the oxygen evolving system is inactivated [26,28]. Thus, the light-minus-dark difference spectrum of the reversible absorbance change seen in Fig. 1 under the conditions used for Trace 2 is typical of $P680^+$ (Fig. 2) [26,28]. It was previously demonstrated that the above conditions also result in the formation of $Z^\bullet$ [23]. Thus, the use of the preparations studied in Fig. 1 (Trace 2) (Mn-depleted PS II particles in the presence of artificial electron acceptors) in the present experiments are expected to lead to the formation of both $Z^\bullet$ and $P680^+$ upon illumination.

These samples were studied using the FTIR technique. In Fig. 3, the IR spectra of the preparations before and during illumination are presented together with the light-minus-dark difference spectrum. In the difference spectrum, the bands that arise from P680 and Z appear as negative absorption changes while the bands that originate from the formation of $P680^+$ and $Z^\bullet$ are seen as positive absorption changes.

In the region below 1650 cm^{-1}, the analysis of the Chl vibrations associated with the formation of $P680^+$ is complicated by the possible superposition of changes in the redox state of cytochrome b_{559}, plastoquinone, tyrosine, or from other amino agroups that might be subjected to some modification of their interacting milieu following photooxidation of Z and P680. The IR spectra

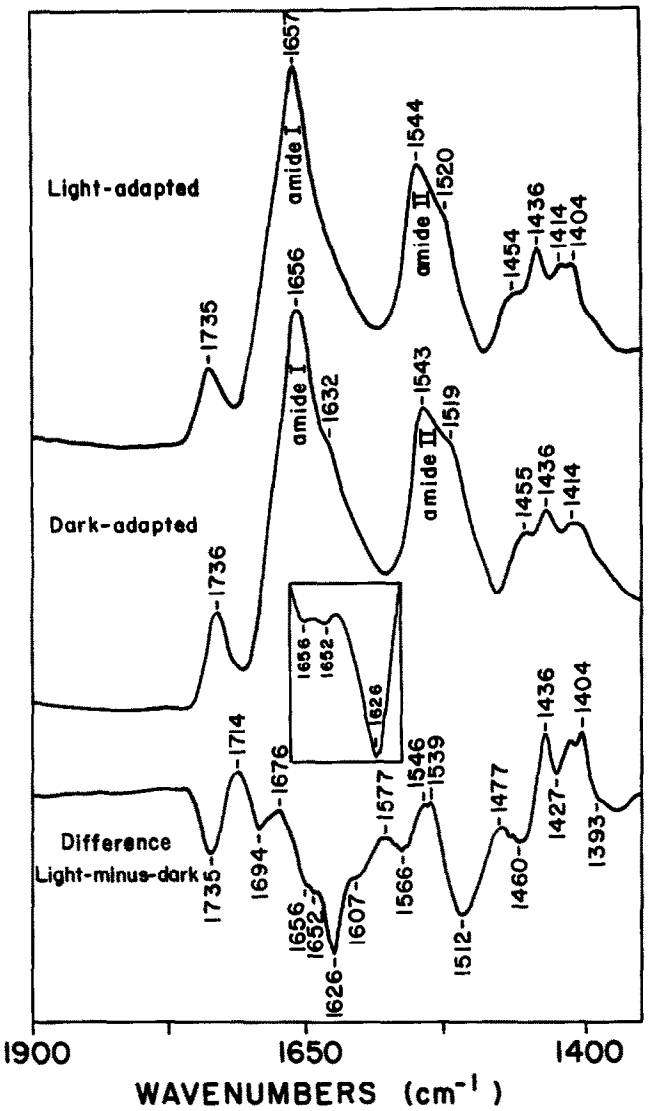

Fig. 3 FTIR spectra of Mn-depleted PS II preparations in the presence of 500 μM potassium ferricyanide and 10 μM silicomolybdate obtained in the dark or during illumination and the resulting light-minus-dark difference spectra. Inset: expanded portion (1620–1650 cm^{-1} region) of the light-minus-dark difference spectra.

of the light- and dark-adapted Mn-depleted particles exhibited no major difference (frequency shifts) in the amide I (1657 cm^{-1}) and amide II (1544 cm^{-1}) domains thus excluding any large conformational changes occurring upon charge separation which confirms the previous observations of Tavitian et al. [17]. Minor differences in the intensities of the amide I and II bands are reflected by shoulder peaks at 1656 and 1546 cm^{-1} in the difference spectrum.

Comparison of the light-minus-dark spectrum of Fig. 3 with the known FTIR spectra does not give any clear evidence for the participation of cytochrome b_{559} or plastoquinone in this spectrum. In fact, in the spectral region studied, cytochrome b_{559} in its reduced form was characterized by vibration modes at 1685, 1673, 1641, 1620, 1545, and 1406 cm^{-1}, and the oxidized form absorbed at 1656, 1628, and 1608 cm^{-1} [22]. This pattern of bands is absent in the difference spectrum. At most, only a few minor bands or shoulders could correspond to the above-mentioned maxima and be assigned to the cytochrome. In the case of plastoquinone, none of the bands associated with its vibration modes [21] are found. In contrast with the study of MacDonald and Barry [19], a positive band at 1514 cm^{-1}, which they assigned to oxidized tyrosine, is not found. Instead, a negative band at 1512 cm^{-1}, corresponding to the loss of intensity of the tyrosine band at 1519 cm^{-1} in the IR spectrum obtained from the illuminated particles, is observed in the difference spectrum together with a negative shoulder at 1607. These vibrations are characteristic of the ring modes of phenol groups that are absent in the tyrosine radicals [29]. The positive band at 1477 cm^{-1} may be tentatively associated with the C-C stretch mode of the tyrosine radical [29]. On the other hand, the positive features centered at 1400–1477 cm^{-1} are related to the C-O stretching and C-H binding modes and can originate from either Chl or tyrosine.

The main and most interesting Chl bands are found in the region between 1735 and 1650 cm^{-1} in the difference spectrum of Fig. 3 where their ester and ketone vibration modes are evidenced. Chlorophyll *a* usually shows two bands in that region. An absorption band originates from its two ester groups at 1735 cm^{-1}, and a peak at 1694 cm^{-1} is due to the vibration mode of a free (not hydrogen-bonded) 9-keto C=O in ring V of the porphyrin [30]. These two bands are found in the difference spectrum as negative features together with a third band at 1652 cm^{-1}, further evidenced in the inset of Fig. 3, that could also be assigned to the 9-keto C=O group. The presence of an absorption band at 1652 cm^{-1} was used as a diagnostic of aggregation interaction between Chl *a* molecules since it originates from coordinated C=O in Chl solutions [30]. The occurrence of the peak at this frequency in the difference spectrum (Fig. 3 and inset) could indicate that P680 is formed by a coordinated Chl dimer. The above would imply the coordination of the 9-keto function from one Chl to the central Mg ion of the other Chl which keeps its 9-keto group free [30]. Alternatively, the strong negative band appearing at 1626 cm^{-1} can also be attributed to a strongly associated 9-keto function [31,32] as similarly assigned in the difference spectrum obtained for triplet P680 [33].

Upon photooxidation of P680, the band at 1694 cm^{-1} that originates from the free 9-keto groups is shifted to higher frequencies and appears as a positive absorption at 1714 cm^{-1} in the difference spectrum as in the case of photooxidation of monomeric Chl *a* or of the P700 Chl in the PSI reaction center complex [34], indicating that this group is now in an environment with even less interaction or with lower dielectric constant [31]. On the other hand, the band at 1652 or the one at 1626 cm^{-1} assigned

to a strongly associated 9-keto group is shifted to 1676 cm^{-1}, a frequency still in the position of an associated ketone (possibly hydrogen-bonded [31,32], but not indicative of a coordination bond. We must note that in the difference spectrum, the absorption peaks cannot be quantitatively evaluated because of the presence of both positive and negative bands. Thus, it is not clear if the band at 1676 cm^{-1} originates from a shift of the band at 1652 or from that at 1626 cm^{-1}.

The occurrence of two pairs of bands attributed to 9-keto groups (1714/1694 cm^{-1} and 1676/1652–1626 cm^{-1}) demonstrates the dimeric nature of P680 in agreement with recent FTIR studies of the triplet state of this reaction center [33]. The above data are also in agreement with the known structure of the bacterial reaction center [9]. The occurrence of a dimeric P680 with the radical of the charge separated state delocalised on one of the Chl could explain the conflicting interpretation of most of the data reported on the structure (monomeric or dimeric) of this reaction center [14].

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References

[1] Hansson, Ö. and Wydrzynski, T. (1990) Photosynth. Res. 23, 131–162.
[2] Yocum, C.F. (1991) Biochim. Biophys. Acta 1059, 1–15.
[3] Homann, P.H. (1987) J. Bioenerg. Biomemb. 19, 105–123.
[4] Renger, G. (1992) in: The PSs:Structure, Function and Molecular Biology (Barber, J., Ed.) pp. 45–99, Elsevier, Amsterdam.
[5] Metz, J.G., Nixon, P.J., Rogner, M., Brudvig, G.M. and Diner, B.A. (1989) Biochemistry 29, 5109–5118.
[6] Boska, M., Sauer, K., Buttner, W. and Babcock, G.T. (1983) Biochim. Biophys. Acta 722, 327–330.
[7] Gerken, S., Brettel, K., Schlodder, E. and Witt, H.T. (1988) FEBS Lett. 237, 69–75.
[8] Trebs, A. (1986) Z. Naturforsch. 41c, 240–245.
[9] Michel, A. and Deisenhofer, J. (1988) Biochemistry 27, 1–7.
[10] Braun, P., Greenberg, B.M. and Scherz, A. (1990) Biochemistry 29, 10376–10387.
[11] Otte, S.C.M., Van der Vos, R. and Van Gorkum, H.J (1992) J Photochem. Photobiol. (B), 5–14.
[12] Telfer, A., Barber, J. and Evans, M.C.W. (1988) FEBS Lett 232, 209–213.
[13] Nugent, J.H.A., Telfer, A., Demetriou, C. and Barber, J. (1989) FEBS Lett. 255, 53–58.
[14] Van der Vos, R., Van Leeuwen, P.J., Braun, P. and Hoff, A.J (1992) Biochim. Biophys. Acta 1140, 184–198.
[15] Tetenkin, V.L., Gulyaev, B.A., Seibert, M. and Rubin, A.B. (1989) FEBS Lett. 250, 459–463.
[16] Shuvalov, V.A., Heber, U. and Schreiber, U. (1989) FEBS Lett. 258, 27–31.
[17] Tavitian, B.A., Nabedryk, E., Mäntele, W. and Breton, J. (1986) FEBS Lett. 201, 151–157.
[18] Noguchi, T., Ono, T.-a. and Inoue, Y. (1992) Biochemistry 31,
[19] MacDonald, G.M. and Barry, B.A. (1992) Biochemistry 31, 9848–9856.
[20] Nabedryk, E., Andrianambinintsoa, S., Berger, G., Leonhard, M., Mäntele, W. and Breton, J. (1990) Biochim. Biophys. Acta 1016, 49–54.
[21] Berthomieu, C., Nabedryk, E., Mäntele, W. and Breton, J. (1990) FEBS Lett. 269, 363–367.
[22] Berthomieu, C., Boussac, A., Mäntele, W., Breton, J. and Nabedryk, E. (1992) Biochemistry 31, 11460–11471.
[23] Ghanotakis, D.F. and Babcock, G.T. (1983) FEBS Lett 153, 231–234.
[24] DePaula, J.C., Innes, J.B. and Brudvig, G.W. (1985) Biochemistry 24, 8114–8120.
[25] Allakhverdiev, S.I. and Klimov, V.V. (1992) Z. Naturforsch. 47c, 57–62.
[26] Allakhverdiev, S.I., Shafiev, M.A. and Klimov, V.V. (1986) Photobiochem. Photobiophys. 12, 61–65.
[27] Klimov, V.V., Allakhverdiev, S.I., Shuvalov, V.A. and Krasnovsky, A.A. (1982) FEBS Lett. 148, 307–312.
[28] Van Gorkum, H.J., Pulles, N.P.J. and Wessels, J.S.C. (1975) Biochim. Biophys. Acta 408, 331–339.
[29] Tripathi, G.N.R. and Schuler, R.H. (1984) J. Chem. Phys 113–121.
[30] Ballschmiter, K. and Katz, J.J. (1969) J. Am. Chem. Soc. 91, 2661–2677.
[31] Chapados, C. (1988) Photochem. Photobiol. 47, 115–131.
[32] Chapados, C., Lemieux, S. and Carpentier, R. (1991) Biophys. Chem. 39, 225–239.
[33] Noguchi, T., Inoue, Y. and Satoh, K. (1993) Biochemistry 32, 7186–7195.
[34] Nabedryk, E., Leonhard, M., Mäntele, W. and Breton, J. (1990) Biochemistry 29, 3242–3247.