Hypothesis

The 695 nm fluorescence (F_{695}) of chloroplasts at low temperature is emitted from the primary acceptor of photosystem II

Jacques Breton

Service de Biophysique, Département de Biologie, CEN Saclay, 91191 Gif-Sur-Yvette, France

Received 23 July 1982

Photosystem *Fluorescence* F_{695} *Chloroplast* *Chlorophyll* *Pheophytin*

1. INTRODUCTION

Fluorescence is one of the earliest and most commonly used methods to explore the primary photophysical and photochemical processes of photosynthesis (reviews [1,2]). The intrinsic heterogeneity in both the structure and the function of the various chlorophyll–protein complexes which constitute the building-blocks of the photosynthetic membrane, as well as the very rapid exchange of energy among these complexes have been revealed in some of the properties of the fluorescence emitted at room temperature [3]. However, this heterogeneity is further shown by the resolution of several bands in the emission spectra of chloroplasts and algae at low temperature. Furthermore, the observation that the separation and stabilization of charges which follow the trapping of the exciton by the reaction center induces characteristic changes in the fluorescence yield [4,5], has led many researchers to use fluorescence spectroscopy at room and at low temperatures as a tool to monitor the primary processes of photosynthesis.

2. SOME CHARACTERISTICS OF THE FLUORESCENCE EMISSION AT LOW TEMPERATURE

The dependence of the fluorescence emission of chloroplasts and algae upon the temperature (300–5 K) has been described in [6–9]. At room temperature the emisson mostly consists in a single broad band centered at 685 nm. Upon cooling, a strong increase in the intensity of a long wavelength emission (710–740 nm) is observed. Then, at ≤ 100 K, an emission band develops around 695 nm and at 77 K a characteristic pattern of 3 bands located near 685, 695 and 735 nm is observed. These bands have been named F_{685}, F_{695} and F_{735}, respectively. Upon further cooling F_{695} increases considerably, until it reaches a plateau at ~ 35 K. Below this temperature, an increase in F_{685} mainly and the development of weak fluorescence bands at shorter wavelengths (F_{680}) ([10] J.B., M. Lutz, unpublished) are noted.

The attribution of these various emission bands to well-defined chlorophyll–protein complexes within the membrane, or to chlorophyll species within these complexes, is still quite speculative. F_{735}, which is actually thought to be of composite nature [11,12], has been associated with photosystem I (PS I). F_{685} has been attributed either to the light-harvesting complex (LHC) [8] or to a photosystem II (PS II) antenna [5]. The latter assignment is reinforced by the observation of a normal F_{685} band in organisms which are deficient in LHC (chlorophyll *b*-less mutant, blue–green algae [9]). Apparently the emission from LHC is very weak, even at 77 K, its presence being detected only by fluorescence polarization [11] or by phase fluorimetry [12]. Actually, only at < 35 K is it possible to attribute an F_{680} emission band to this complex [13]. The origin of F_{695} is even more controversial. It was first attributed by Govindjee [14] and by Bergeron [15] in 1963 to the trap of PS II and was later more precisely assigned to P680, the

Published by Elsevier Biomedical Press

00145793/82/0000–0000/$2.75

primary donor of PS II [6]. Butler [8], while developing his bipartite and tripartite models of the photosynthetic apparatus, assumed that F_{695} originated directly from the antenna bed of PS II, but later proposed that this fluorescence was emitted from energy trapping center(s) appearing below 100 K in PS II. An identical proposal was also made by Rijgersberg et al. [16].

It should be noted that F_{695} exhibits an unusual anisotropic emission property which was first reported by Garab and Breton [11] and later confirmed by Vasin and Verkhoturov [17]. In contrast to all of the other emission bands detected at low temperature (F_{680}, F_{685}, F_{735}) which arise from transitions lying in or close to the membrane plane, the F_{695} component originates from transitions orientated preferentially perpendicular to the membrane plane. This is especially noticeable in [17] where the magnitude of the F_{695} component is always stronger for the polarization perpendicular to the membrane plane than parallel to it, an observation which has been confirmed by fluorescence studies at 30–50 K on magnetically oriented chloroplasts (J.B., M. Lutz, unpublished).

3. THE ORIGIN OF THE FLUORESCENCE OF VARIABLE YIELD

Role and orientation of primary acceptor of PS II

The fluorescence yield of chloroplasts is a function of the state of the trap of PS II [1,2]. In the open state of the reaction center the fluorescence yield is small (F_0). Upon trapping of an exciton, the reaction center assumes a closed state in which useful photochemistry is no longer possible and in which the fluorescence yield increases by a factor of ~5 at room temperature. This increase (called induction) of the fluorescence yield has been correlated by Duysens and Sweers [4] with the redox state of the first stable electron acceptor Q (a bound quinone molecule) in the reaction center. The evidence for an induction phase of F_{685} and F_{695} even at 5 K [9] demonstrates that some charge separation and stabilization can occur at any temperature. In [18] Klimov et al. detected by absorption and EPR spectroscopies a very early acceptor which functions between P680 and Q. This acceptor has been identified as a pheophytin *a* molecule (Phe) and it has been proposed that at room temperature the variable fluorescence (F_v) results from charge recombination between $P680^+$ and Phe^-, occuring in the state $P680^+$ Phe^- Q^-, and which repopulates the excited singlet state of chlorophyll [19]. In line with this interpretation was the observation of a large quenching of the fluorescence at room temperature [19] when the reaction center is in the state P680 Phe^- Q^- (a state which can be accumulated upon strong illumination of chloroplasts at low redox potential).

Most recently the orientation of this primary acceptor Phe has been investigated in oriented PS II particles. By measuring the dichroism of the absorption changes linked to the reduction of Phe, Ganago et al. [20] have shown that its Q_y transition (and consequently the plane of its macrocycle) was rather perpendicular to the largest dimension(s) of the particle. This conclusion has been independently confirmed by the study of a variety of PS II particles [21,22]. Furthermore by demonstrating that the largest dimensions of all these PS II particles in vivo are lying parallel to the plane of the thylakoid [23], it can be unambiguously concluded that in the intact membranes the Q_y transition of the primary acceptor is preferentially oriented perpendicular to the membrane plane [21,23]. On the other hand, we have recently investigated the linear dichroism at 100 K of the central core of PS II [21] using particles containing ~20 chlorophyll molecules/P680 [24]. We have determined that the Q_y transitions of the PS II chlorophyll *a* were oriented in such a way that in the intact membrane they are lying mostly parallel to the membrane plane (J.B., P. Tapie, B. Diner, in preparation).

4. THE HYPOTHESIS, ITS LIMITATIONS AND ITS IMMEDIATE IMPLICATIONS

In the past 10 years the orientation of pigments in photosynthetic systems (intact membranes, isolated complexes) has been extensively investigated using a variety of optical techniques (review, [25]). It has been demonstrated [23,25–28] that in thylakoids most, if not all, of the pigments were oriented to some extent with respect to the membrane plane. All of the dichroic long-wavelength transitions of chlorophyll *a* molecules which we have investigated have turned out to be lying preferentially parallel to the plane of the membrane. This is especially true for the antenna transitions absorbing and fluorescing at the long wavelengths

[11,28] and for the primary donors of PS I (P700) [29,30] and PS II (P680) [31]. We have already proposed [32,33] that this geometry, which funnels the exciton in the plane of the membrane, might constitute a way of increasing the efficiency of capture of this exciton by the reaction centers. In this respect, the hypothesis that F_{695} originates from energy trapping centers observed upon lowering the temperature [8] seems at odds with the orientation perpendicular to the membrane plane of its Q_y transition moment.

Accordingly, and extrapolating to low temperature the hypothesis of Klimov on the origin of the variable fluorescence [19], and using the orientation perpendicular to the membrane plane of both the Q_y transition responsible for F_{695} and of the Q_y transition of Phe as circumstantial evidence, we propose to identify F_{695} as the fluorescence emitted directly from the Phe partner of the P680–Phe pair when the $P680^+$–Phe^- charges recombine. In other words, F_{695} represents the photon emitted when the singlet exciton generated by the charge recombination becomes localized on Phe and decays to the ground state. Although, at this writing, our data cannot totally exclude the possibility of the presence of one additional PS II chlorophyll *a* molecule oriented with the same geometry as Phe, and which would account for the F_{695} emission, we see no heuristic value in considering this rather improbable situation.

Although this hypothesis on the molecular origin of F_{695} is at variance with that of Butler [8], the models which he has proposed for the energy flow in the photosynthetic apparatus should not be affected by this reassignment as long as F_{695} samples the quenching state of the PS II reaction centers. Following Haehnel et al. [34], we visualize the energy flow in the photosynthetic apparatus at room temperature as follows: excitons deposited in the LHC bed become rapidly partitioned between PS I (trapping time 50–100 ps) and the antenna system of PS II with a trapping time of ~500 ps, which is rather independent of the state of the reaction center (q or Q^-). However, each time an exciton becomes trapped by a reaction center already in the state P680 Phe Q^- the back reaction between $P680^+$ and Phe^- regenerates an exciton which returns to the PS II antenna. The 1–2 ns lifetime observed for this emission must comprise the rate constant for the back reaction and the possibility for this exciton to visit more than one PS II unit. We interpret the increase in F_{695} (both F_o and F_v) observed upon lowering the temperature below 100 K [9] as due to an increasing localization on Phe of the exciton created by the charge recombination and which at room temperature would otherwise be in equilibrium with the PS II antenna.

Before attempting to use this model to explain some known properties of F_o and F_v for both F_{685} and F_{695} as a function of temperature [9] or of various physical or chemical treatments in terms of trapping, charge stabilization, back reaction and emission from either Phe or P680 or the PS II antenna bed, we believe that it would be more useful to try to confirm the hypothesis proposed.

5. HOW TO VERIFY THE PROPOSED HYPOTHESIS

While developing this hypothesis we have deliberately avoided taking into highly significant account the low temperature fluorescence emission spectra of subchloroplast fractions. Abundant literature exists on the subject [9,13,35] and some F_{695} has been observed in PS II particles. However, we believe that the fluorescence spectra of such fractions can be misleading. Indeed, in contrast to its absorption properties, the fluorescence emission spectra of a detergent-treated system can be dominated by the contribution of a small number of pigments which may have been partially disorganized during the solubilization and purification processes. Furthermore, this effect will tend to be most important in those systems which have a low fluorescence yield in the intact membrane.

The hypothesis that F_{695} originates from Phe implies that the state P680 Phe^- Q^- of the reaction center should act as a quencher of F_{695} at low temperature. Accordingly, we have illuminated chloroplasts and PS II particles (in the states Q or Q^-) at room and at low temperature in order to trap the state P680–Phe^- Q^- [19]. At 77 K, we have observed a large quenching of F_{695} and F_{685} when the state P680 Phe^- is present (J. B., A. W. Rutherford, unpublished), in agreement with our proposed scheme. However, a model based upon circumstantial evidence is often difficult to verify directly and, although this test indicates a link between F_{695} and Phe, it does not prove the valid-

ity of our hypothesis. Another way to further test the validity of this hypothesis is to compare on the same oriented chloroplasts the tilt angles of the Q_y transitions of the Phe molecule and of the species emitting at 695 nm.

This hypothesis, which might also prove to be useful in explaining the true molecular origin of the F_{685} fluorescence at low temperatures (PS II antenna fluorescence or emission arising directly from P680 upon charge recombination), may help in obtaining a better understanding of the trapping of excitons. In this regard it must be noticed that in PS II, this rather special molecular pair (P680–Phe) comprises a donor whose Q_y transition is oriented in the membrane plane, like the Q_y transitions of the antenna chlorophylls, while the acceptor has its Q_y transition moment oriented perpendicular to this plane. A similar geometry has also been observed in bacterial systems [25,36,37]. Such a geometry will most probably favor a trapping of the exciton by the primary donor rather than by the acceptor. This 'polarity', as well as the perpendicular geometry of the two partners, which precludes easy transfer of the excitation energy, might play some role in the transformation of this energy from the singlet excited state possibly to triplet states and ultimately to the biradical state of the pair. In turn, such a geometry would also help to stabilize these charges for efficient photosynthesis.

ACKNOWLEDGEMENTS

I wish to acknowledge many stimulating discussions within the Photosynthesis Group of Saclay. Furthermore the presence in this group of Professor N.E. Geacintov and Dr A.W. Rutherford has been of great value in the formulation and in the early tests of the model, respectively.

REFERENCES

[1] Butler, W.L. (1978) Annu. Rev. Plant Physiol. 29, 345–378.
[2] Lavorel, J. and Etienne, A.L. (1977) in: Primary Processes of Photosynthesis. (Barber, J. ed) Top. Photosynth. vol. 2, pp. 203–268, Elsevier Biomedical, Amsterdam, New York.
[3] Lavorel, J. (1962) Biochim. Biophys. Acta 60, 510–523.
[4] Duysens, L.N.M. and Sweers, H.E. (1963) in: Studies on Microalgae and Photosynthetic Bacteria; spec. iss. Plant Cell Physiol. (Jap. Soc. Plant Physiol. ed) pp. 353–372, Univ. of Tokyo Press, Tokyo.
[5] Murata, N. (1968) Biochim. Biophys. Acta 162, 106–121.
[6] Cho, F. and Govindjee (1970) Biochim. Biophys. Acta 205, 371–378.
[7] Cho, F. and Govindjee (1970) Biochim. Biophys. Acta 216, 139–150.
[8] Butler, W.L. (1978) in: Chlorophyll Organization and Energy Transfer in Photosynthesis, Ciba Found. Symp. 61 (new ser.) pp. 305–322, Elsevier/Excerpta Medica, Amsterdam, New York.
[9] Rijgerberg, C.P. (1980) Thesis, University of Leiden.
[10] Amesz, J. and Rijgersberg, C.P. (1981) in: Proc. 5th Internat. Cong. Photosynthesis, 1980, Halkikiki (Akoyunoglou, G. ed) pp. 739–747 Balaban Int. Sci. Services, Philadelphia PA.
[11] Garab, G. and Breton, J. (1976) Biochem. Biophys. Res. Commun. 71, 1095–1102.
[12] Moya, I. (1979) Thesis, Université de Paris Sud.
[13] Rijgersberg, C.P., Amesz, J., Thielen, A.P.G.M. and Swager, J.A. (1979) Biochim. Biophys. Acta 545, 473–482.
[14] Govindjee (1963) in: Photosynthetic Mechanisms of Green Plants, Natl. Acad. Sci. USA, Natl. Res. Council Publ. 1145, 318–334.
[15] Bergeron, J.A. (1963) in: Photosynthetic Mechanisms of Green Plants, Natl. Acad. Sci. USA Natl. Res. Council Publ. 1145, 527–536.
[16] Rijgesberg, C.P., Melis, A., Amesz, J. and Swager, J.A. (1978) in: Chlorophyll Organization and Energy Transfer in Photosynthesis, Ciba Found. Symp. 61 (new ser.) pp. 305–322, Elsevier/Excerpta Medica, Amsterdam, New York.
[17] Vasin, Y.A. and Verkhoturov, V.N. (1979) Biophysics 24, 269–273.
[18] Klimov, V.V., Klevanik, A.V., Shuvalov, V.A. and Krasnovsky, A.A. (1977) FEBS Lett. 82, 183–186.
[19] Klimov, V.V., Ke, B. and Dolan, E. (1980) FEBS Lett. 118, 123–126.
[20] Ganago, I.B., Klimov, .V., Ganago, A.O., Shuvalov, V.A. and Erokhin, Y.E. (1982) FEBS Lett. 140, 127–130.
[21] Breton, J., Tapie, P., Acker, S. and Diner, B. (1982) Congr. Soc. Chim. Biol. 9.
[22] Tapie, P., Acker, S., Diner, B. and Breton, J. (1982) 10th Annu. Meet. Am. Soc. Photobiol., Vancouver, abst. PS 1–5.
[23] Tapie, P., Haworth, P., Hervo, G. and Breton, J. (1982) Biochim. Biophys. Acta in press.
[24] Wollman, F.A. (1982) Thesis, Université de Paris VII.

[25] Breton, J. and Verméglio, A. (1982) in: Photosynthesis: Energy Conversion by Plants and Bacteria (Govindjee ed) Academic Press, New York, in press.
[26] Haworth, P., Arntzen, C.J., Tapie, P. and Breton, J. (1982) Biochim. Biophys. Acta 679, 428–438.
[27] Haworth, P., Tapie, P., Arntzen, C.J. and Breton, J. (1982) Biochim. Biophys. Acta in press.
[28] Becker, J.F., Breton, J., Geacintov, N.E. and Trentacosti, F. (1976) Biochim. Biophys. Acta 440, 531–544.
[29] Breton, J., Roux, E. and Withmarsh, J. (1975) Biochem. Biophys. Res. Commun. 64, 1274–1277.
[30] Breton, J. (1977) Biochim. Biophys. Acta 459, 66–75.
[31] Mathis, P., Breton, J., Verméglio, A. and Yates, M. (1976) FEBS Lett. 63, 171–173.
[32] Breton, J. and Geacintov, N.E. (1978) in: Chlorophyll Organization and Energy Transfer in Photosynthesis, Ciba Found. Symp. 61 (new ser.) pp. 217–236, Elsevier/Excerpta Medica, Amsterdam, New York.
[33] Breton, J. (1977) Thesis, Université de Paris Sud.
[34] Haehnel, W., Nairn, J.A., Reisberg, P. and Sauer, K. (1982) Biochim. Biophys. Acta 680, 161–173.
[35] Thornber, J.P., Markwell, J.P. and Reinman, S. (1979) Photochem. Photobiol. 29, 1205–1216.
[36] Verméglio, A. and Clayton, R.K. (1976) Biochim. Biophys. Acta 449, 500–515.
[37] Paillotin, G., Verméglio, A. and Breton, J. (1979) Biochim. Biophys. Acta 545, 249–264.