Biochimica et Biophysica Acta, 1099 (1992) 81–84
© 1992 Elsevier Science Publishers B.V. All rights reserved 0005-2728/92/$05.00

BBABIO 43544

The fluorescence yield of *Rhodopseudomonas viridis* in relation to the redox state of the primary electron donor

Geurt Deinum, Frank A.M. Kleinherenbrink, Thijs J. Aartsma and Jan Amesz

Department of Biophysics, Huygens Laboratory, University of Leiden, Leiden (The Netherlands)

(Received 28 June 1991)

Key words: Fluorescence; Bacteriochlorophyll; Primary electron donor; Redox state; (*Rps. viridis*)

The fluorescence yield of bacteriochlorophyll (BChl) *b* in membranes of *Rhodopseudomonas viridis* was measured immediately and at a variable time-interval after a saturating laser flash to bring about charge separation. At 4 K a decrease of the yield by 28% was observed immediately after the flash. This yield recovered mono-exponentially with a time constant of 6.3 ± 0.4 ms to approximately the original level. The same time constant was observed for the re-reduction of the primary electron donor, indicating that the fluorescence quenching can be ascribed to the oxidation of the primary donor. The extent of quenching decreased with increasing temperature and reversed to a fluorescence increase at temperatures above 50 K. These results may be explained by the presence of long-wavelength absorbing BChls *b* in the antenna which at low temperature transfer their excitation energy more efficiently to the oxidized than to the reduced primary donor, in support of a similar hypothesis used to explain the quenching of fluorescence by 'oxidized' reaction centers in *Heliobacterium chlorum* (Deinum, G., Kramer, H., Aartsma, T.J. Kleinherenbrink, F.A.M. and Amesz, J. (1991) Biochim. Biophys. Acta 1058, 339–344)

Introduction

Energy transfer from the antenna bacteriochlorophylls (BChls) to the primary electron donor in the reaction center presumably takes place by means of the Förster mechanism [1]. Since the absorption of the oxidized primary donor is generally less than that of the reduced donor, the Förster overlap integral will decrease upon charge separation and, because excitations can now no longer be transferred efficiently to the reaction center, the fluorescence of BChl will increase [2].

Recently, however, we found that in *Heliobacterium chlorum* the fluorescence decreases after charge separation [3]. If one assumes that the Förster mechanism applies for transfer to both the reduced and the oxidized primary donor, one has to conclude that in this case the Förster overlap increases. We suggested that this may be due to the presence of antenna BChls with excited state energy levels lower than that of the reduced primary donor. These BChls then would have a relatively small Förster overlap with the donor, which overlap might well increase upon oxidation.

This paper deals with experiments with the BChl-*b*-containing purple bacterium *Rhodopseudomonas viridis*. The primary electron donor, P-985, shows an absorption maximum at 985 nm [4], while that of the antenna is observed at 1015 nm. It will be shown that, as in *H. chlorum*, the fluorescence yield decreases at low temperature upon photo-oxidation of the primary electron donor. This supports the hypothesis that the fluorescence quenching observed upon oxidation of the reaction centers may be caused by an enhanced Förster overlap.

Materials and Methods

Rhodopseudomonas viridis was grown as described in Ref. 5. Membrane fragments were prepared by 10 min sonication and centrifugation according to Ref. 6. The membranes were suspended in a buffer containing 20 mM Tris (pH 8.0) and mixed with 50% glycerol (v/v) to prevent freezing when stored at −20°C.

The apparatus used for the fluorescence measurements was described elsewhere [7]. The wavelength of

Abbreviations: BChl, bacteriochlorophyll; P-985, primary electron donor.

Correspondence: J. Amesz, Department of Biophysics, Huygens Laboratory, University of Leiden, P.O.Box 9504, 2300 RA Leiden, The Netherlands.

the exciting laser pulse was 532 nm with a pulse length of approx. 25 ps. The relative yield of fluorescence was monitored by measuring the amount of fluorescence induced by a weak 10 μs xenon flash, given at a variable delay time after the flash. The light of the xenon flash was filtered by means of a combination of filters to a 40 nm wide band centered at 515 nm. A helium flow cryostate was used for the experiments at low temperature. The absorbance of the samples at 532 nm was kept low (< 0.03) in order to achieve a homogeneous light distribution within the sample. The fluorescence passed a monochromator set at a bandwidth of 3 nm and was measured with an integrating photodiode (RCA-30810). Absorbance difference measurements were performed as described by Smit et al. [8].

Results

A suspension of membranes of *Rps. viridis* was cooled to 4 K and excited by a strong, saturating, 25 ps laser flash to bring about charge separation. At a variable delay after the laser flash a weak xenon flash was used to monitor the fluorescence. Fig. 1 shows the relative fluorescence due to the xenon flash as a function of the delay between the two flashes. The fluorescence was normalized to the fluorescence intensity in the absence of a preceding laser flash. The results show that, immediately after the saturating laser flash, the fluorescence decreased to 72% of its original level. With increasing delay time the fluorescence increased again and reached approximately its original level. A mono-exponential decay time of 6.3 ± 0.4 ms was determined for this recovery. Measurements on the same sample of the rate of re-reduction of P-985$^+$ by means of absorption difference measurements, as shown in Fig. 1 (inset), gave similar kinetics (6.0 ± 0.5 ms), indicating that the quenching of fluorescence induced by the laser flash can be ascribed to the formation of P-985$^+$.

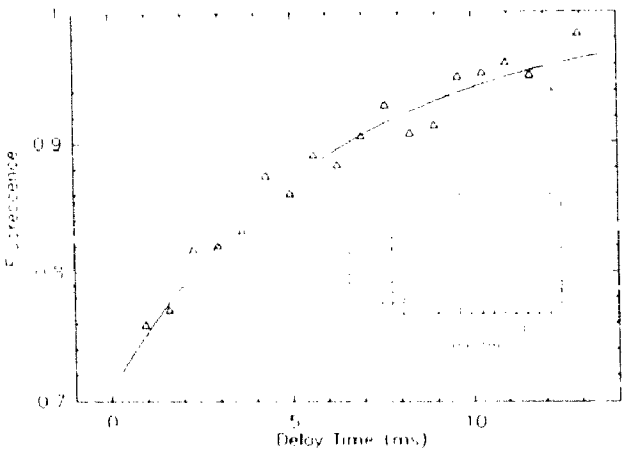

Fig. 1. Relative fluorescence at 1060 nm of membranes of *Rps. viridis*, measured at 4 K with a weak xenon flash at a variable time after a 25-ps saturating laser flash. The fluorescence was normalized to the fluorescence without preceding laser flash. The energy density of the laser flash was approx. 1 mJ/cm^2. The curve represents a mono-exponential simulation to the data with a decay time of 6.3 ms and an amplitude of 0.28. Inset: Kinetics of P-985$^+$ re-reduction, measured by means of the absorbance change at 840 nm at 4 K, induced by a 532 nm, 15 ns laser flash.

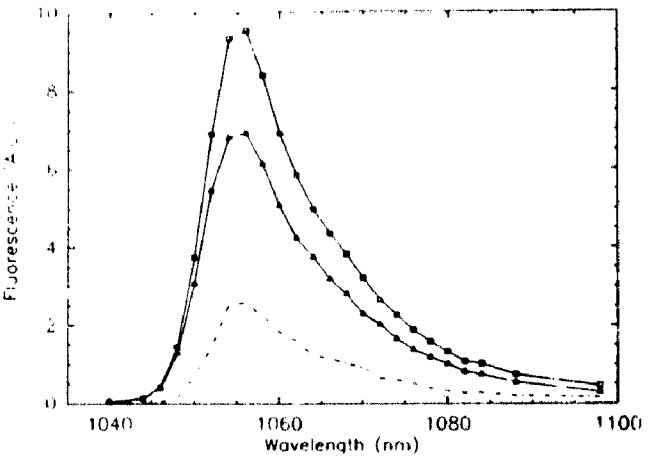

Fig. 2. Fluorescence emission spectra at 4 K measured directly after the laser flash (triangles) and without preceding laser flash (squares). The energy density of the laser flash was about 3 mJ/cm^2. The difference between the two spectra is given by the broken line.

Fig. 2 shows the fluorescence spectra measured at 4 K, with or without preceding laser flash. Apart from differences in intensity, the shapes of the two spectra are very similar. However, inspection of the difference spectrum reveals small deviations at the blue side of the band, indicating that the fluorescence spectrum contains a minor, short wavelength component which is not quenched, or only to a lesser extent, by P-985$^+$. The same effect was observed in *H. chlorum*, where it was more prominent [3].

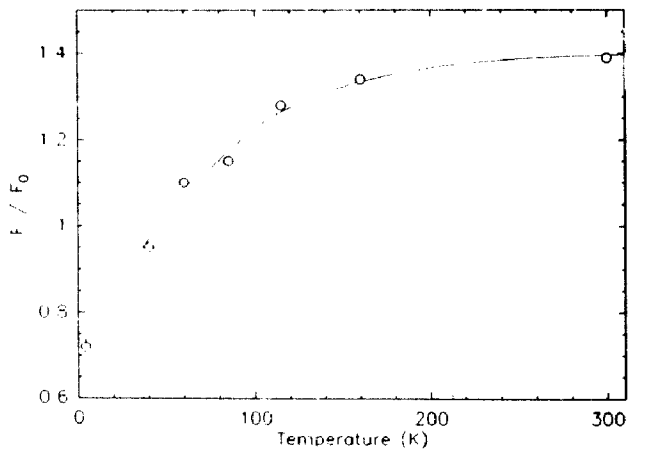

Fig. 3. Temperature dependence of the fluorescence with P-985 oxidized (F) divided by the fluorescence with P-985 reduced (F_0). The fluorescence was measured at 1060 nm, except at 300 K (1045 nm); other conditions as for Fig. 2.

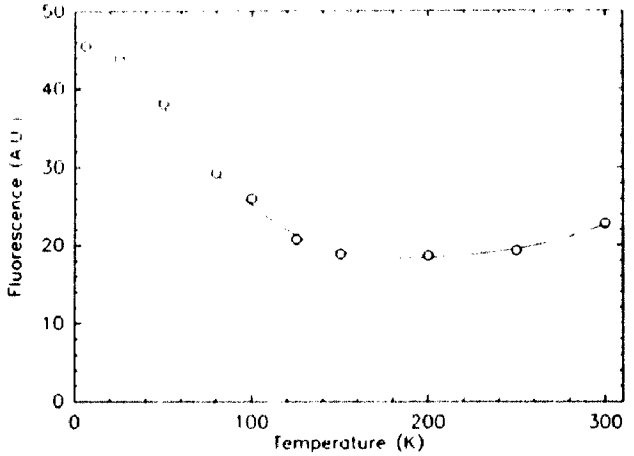

Fig. 4. Temperature dependence of the relative fluorescence yield, integrated over the emission band. Excitation by weak continuous illumination at 605 nm (bandwidth 15 nm). The number of absorbed photons was constant within 10% between 4 and 300 K.

At room temperature the fluorescence yield of *Rps. viridis* membranes has been reported to increase upon photo-oxidation [9] as in other species of purple bacteria [2]. We therefore studied the temperature dependence of the light-induced quenching. The results are shown in Fig. 3. As can be seen, the relative fluorescence yield, F/F_0, measured after the laser flash increases rapidly with temperature. F/F_0 became larger than 1 above 50 K and reached a constant value of 1.4 above 200 K, in agreement with earlier measurements at room temperature [9]. The integrated yield of fluorescence obtained by weak continuous illumination showed a roughly complementary temperature dependence (Fig. 4) with an initial decrease followed by a plateau at higher temperatures.

Discussion

The quenching properties of reaction centers in *Rps. viridis* membranes resemble those of *H. chlorum*, earlier reported [3]. In both organisms the fluorescence at low temperature is quenched more strongly by the oxidized than by the reduced primary electron donor, and in both organisms the relative quenching by the oxidized donor decreases with increasing temperature. In *Rps. viridis* the quenching reverses above about 50 K, in contrast to *H. chlorum*, where it can be observed even at room temperature. It should be noted that in the BChl *a* containing bacteria *Rhodospirillum rubrum* and *Rhodobacter sphaeroides* the yield of antenna fluorescence increases upon photo-oxidation of the primary electron donor even at liquid helium temperature [10].

As mentioned already, we chose *Rps. viridis* as the object of our present study, because in this species, as in heliobacteria, a considerable amount of antenna absorption occurs at longer wavelengths than that of the Q_y-band maximum of the primary electron donor. In membrane fragments of *Rps. viridis* the absorption difference spectrum of P-985 photo-oxidation shows a minimum at 985 nm [4] at room temperature, while the antenna BChl *b* absorption has a maximum at 1015 nm; at liquid helium temperatures the latter maximum shifts to 1040 nm, whereas the triplet minus singlet spectrum of P-985 shows a negative band near 1005 nm [11]. This means that there is a gap of about 300 cm^{-1} between the energy levels of the antenna and the primary electron donor at room temperature as well as at low temperature. As noted already by Holt and Clayton [4], this raises some questions concerning the mechanism and efficiency of energy transfer to the reaction center, especially at low temperature, where the Förster overlap between antenna fluorescence and P-985 absorption is very small [11].

It thus appears that the results obtained with *Rps. viridis* support the hypothesis [3] that the relatively strong quenching by the 'oxidized' reaction centers may be explained by a better overlap with the absorption band of the oxidized than of the reduced primary electron donor. This does not, however, automatically lead to the conclusion that the transfer of energy to P-985 takes place from the same energy level as that to P-985*. It has been suggested [11] that energy transfer to P-985 occurs from a vibronically excited S_1-state of BChl *b*. Alternatively, it might occur from a BChl *b* species absorbing at somewhat shorter wavelength than the pigments responsible for the bulk of the fluorescence emission at low temperature. It should be noted, however, that recent measurements of the action spectrum of charge separation at low temperatures do not appear to support this hypothesis [12]. The temperature dependence of the fluorescence yield (Fig. 4) may be explained by an increasing overlap between BChl *b* fluorescence and P-985 absorption with temperature, caused by a broadening of the corresponding fluorescence and absorption bands. We have no direct information on the extent of overlap with the absorption band of P-985*, but comparison of Figs. 3 and 4 shows that the fluorescence yield is approximately independent of the temperature. This suggests that there is only little change of the Förster overlap, as is not unexpected, since the absorption of P-985* has been reported to extend well into the infra-red [4].

Acknowledgements

The authors wish to thank Prof. R. van Grondelle for critically reading the manuscript. The investigation was supported by the Netherlands Foundation for Biophysics, financed by the Netherlands Organization for the Advancement of Research (NWO) and by the European Community (contract No. SCI*0004-C).

References

1 Van Grondelle, R. (1985) Biochim. Biophys. Acta 811, 147–195
2 Vredenberg, W.J. and Duysens, L.N.M. (1963) Nature 197, 355–357
3 Deinum, G., Kramer, H., Aartsma, T.J., Kleinherenbrink, F.A.M. and Amesz, J. (1991) Biochim. Biophys. Acta 1056, 339–344
4 Holt, A.S. and Clayton, R.K. (1965) Photochem. Photobiol. 4, 899–911
5 Cohen-Bazire, G., Sistrom, W.R. and Stanier, R.Y. (1957) J. Cell Comp. Physiol. 49, 25–68
6 Jay, F., Lambollotte, M. and Muhlethaler, K. (1983) Eur. J. Cell Biol. 30, 1–8
7 Bakker, J.G.C., Van Grondelle, R. and Den Hollander, W.T.F. (1983) Biochim. Biophys. Acta 725, 508–518
8 Smit, H.W.J., Amesz, J. and Van der Hoeven, M.F.R. (1987) Biochim. Biophys. Acta 893, 232–240
9 Trissl, H.W., Breton, J., Deprez, J., Dobek, A. and Lavergne, W. (1990) Biochim. Biophys. Acta 1015, 322–333
10 Vos, M., Van Grondelle, R., Van der Kooij, F.W., Van de Poll, D., Amesz, J. and Duysens, L.N.M. (1986) Biochim. Biophys. Acta 850, 501–512
11 Den Blanken, H.J., Jongenelis, A.P.J.M. and Hoff, A.J. (1983) Biochim. Biophys. Acta 725, 472–482
12 Kleinherenbrink, F.A.M., Deinum, G., Otte, S.C.M., Hoff, A.J. and Amesz, J. (1992) Biochim. Biophys. Acta 1099, in press