Dielectric Relaxation on a Picosecond Time-scale at 77K; a time-resolved Stark fluorescence study on the LH2 B800-820 complex of *Rps. acidophila*

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1.Introduction

The technique of electric field induced (Stark) absorbance and fluorescence difference spectroscopy has proven to be very successful in accessing the properties of the excited states of photosynthetic complexes [1]. The framework for interpreting the Stark effect as it has been developed for the bacterial reaction center [2] has been shown to be also useful for bacterial Light Harvesting complexes[3,4]. The B850 BChl's of LH2 complexes are all characterised by a high difference dipole moment and giant difference polarizability between ground and exited state. Also a considerable amount of loss of dipole strength has been found [3].

2.Materials and methods

All measurements were performed at 77K with samples emerged between 100 um thickness semi-conducting glass plates (the Starkcell). The sample was diluted with a 60% w/v glycerol buffer to obtain a good glass.
The measurements were performed on an integrated set-up for steady state absorption and fluorescence, Stark absorption and fluorescence and time-resolved Stark fluorescence measurements. The time-resolved Stark fluorescence set-up is a combination of a time resolved fluorescence setup with a Streakcamera as detector and a high repetition rate amplified Ti:sapphire laser system as excitation source. The Streak camera has two perpendicular electron deflection plates: vertical: triggered by Ti:sapphire oscillator: time window and horizontal: controlled by the High Voltage generator which also generates the electric field applied over the sample. The horizontal Streak field and the Stark field where synchronized, so the horizontal sweep field follows the Stark field over the sample. The fluorescence was measured through bandpass or coloured glass filters. Excitation pulses of ~ 150 fs @ 514 nm were generated at a 250 kHz repetition rate using a Titanium:sapphire based oscillator (Coherent MIRA SEED) a regenerative amplifier (Coherent REGA) and a a double pass optical parametric amplifier (OPA-9400, Coherent). The flu-

orescence was detected at right angle to the excitation using a Hamamatsu C5680 synchroscan streak camera with an S25 photocathode. The streak-images were recorded on a Hamamatsu C4880 CCD camera which was cooled to -55^{o}C. The full width at half maximum (FWHM) of the instrumental timeresponse was 3ps.

3.Results and Discussion

Steady state measurements

The 77K absorption and fluorescence spectra are shown in fig 1 (left panel, absorption solid, fluorescence dash). The fluorescence maximum is shifted by 22 nm compared to the absorption maximum of the B820 band (resp 824 nm and 846 nm). The sample was excited with a thungsten-halogen lamp with an 514 IF filter for the fluorescence, detection with a monochromator. The fluorescence and Stark fluorescence (steady state) are shown in fig1 (right panel). The Stark fluorescence shows a decrease of approximately 3% relative to the fluorescence maximum. The minimum of the ΔF spectrum is 6 nm blue shifted compared to the fluorescence maximum (resp. 840 nm and 846 nm). The blueshift and lineshape are similar to what is reported for similar measurements on the B800-850 complex of *Rb. sphaeroides* [5] but the magnitude of the effect is two times bigger. Interestingly also the difference polarizability is two times higher for the B850 band of ILH2 complexes of *Rps. acidophila* compared to *Rb. sphaeroides*.

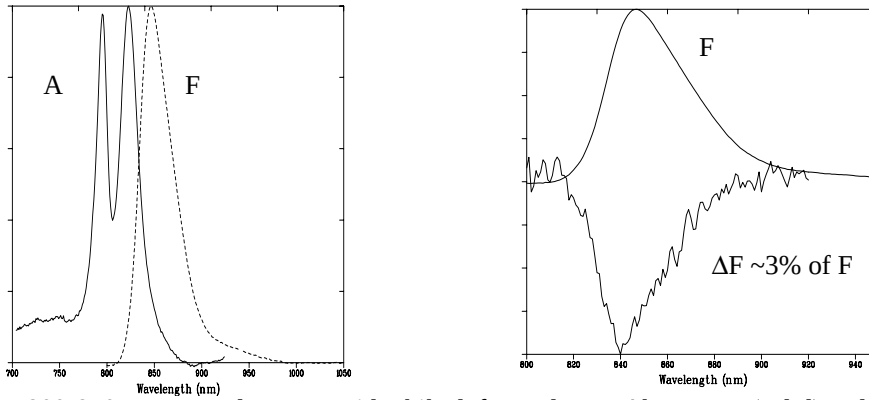

fig 1. B800-820 LH2 complex *Rps. acidophila*; left panel: 77K Absorption (solid) and fluorescence (dash). right panel: fluorescence (top) and Stark fluorescence (bottom). Field : 0.65 MV/cm

The Stark fluorescence effect is one or two orders of magnitude greater than what is normally found for Stark absorption measurements. The effect arises from either a field induced quencing mechanism (field induced coupling of the excited state to a Charge Transfer state) or due to a field effect on the radiative rate. Supporting the latter view are Stark absorption measurements on these and similar LH complexes showing all a considerable amount of loss of absorption dipole strength in the Stark spectra [3]

Time-resolved Stark fluorescence measurements

With use of the horizontal sweeping plates of a synchroscan streak camera on which the same field is applied (but with lower voltage) as on the Starkcell we were able to follow the field effect on the fluorescence during the entire 1.8 ns fluorescence lifetime. (time resolution ranging from 2 ps to 5 ns). Detection is done with a CCD camera which results in a spectrum with a horizontal field strength axis and a vertical time axis. Wavelength selection is performed with the use of filters. The total time and field integrated spectrum is shown in fig 2. (excitation 514 nm, detection >850 nm, field over sample: 0.65 MV/cm). The horizontal sweeping field and the field on the Starkcell were phase matched so most of the signal is on the sides of the spectrum (there the field turns sign so more electrons are deflected there. This was chosen because we need a high S/N in order to see 2-3% effects).

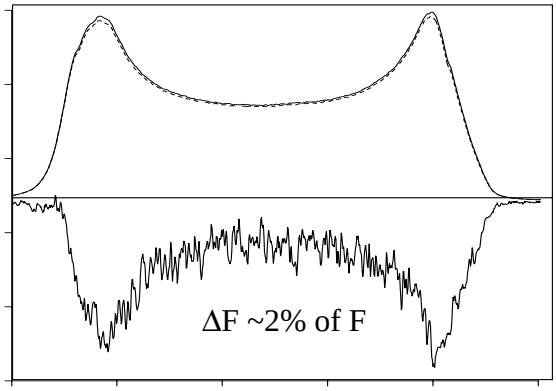

fig 2. total time (10 ps - 1.5 ns) integrated spectrum, upper panel: solid line no field over the sample, dashed with field. Lower panel: difference Field off minus field on.

Mind that the horizontal sweeping field is always on while we switch the field over the sample on and off.

Integrating subsequent slices of the spectrum gives the time dependence of the electric field effect. When we relate the difference in fluorescence ΔF to the total fluorescence F we see a gradual increase for ΔF/F (table 1. We observe that the electric field effect is increasing in time. Measuring at a short time range from 0 - 100 ps results in a $\Delta\mathbf{F}/\mathbf{F}$ of 0.5-0.9%. longer time range: 1.6 - 2 ns: $\Delta\mathbf{F}/\mathbf{F}$ = 5% and even longer >4 ns: ~5%. On the average we see that the time-dependent Stark effect averages to the steady state value but that the effect is not instantaneous. After approximately 1.5 ns we don't observe an increase anymore.

Measurements with a lower electric field shows the same characteristics but with the lower S/N the details are obscured. Detection with an interference filter under different angles didn't show any significant difference. The increase of the electric field effect is what one expects when dielectric relaxation processes take place: regarding the BChl's as in a pigment cavity surrounded by protein residues results in an increase of the electric field in the cavity due to reorienting polar or charged protein residues due to the excited state dipole

moment.)

Table 1: time dependency of ΔF

Time interval	**ΔF/F x** 100%
0 - 124 ps	**0.9%**
124 - 372 ps	**1.2%**
372 - 800 ps	**1.4%**
800 -1160 ps	**1.9%**
1160-1612 ps	**2.5%**

4.Conclusions

It was proposed [5] that the field induced reduction in fluorescence is due to the field induced coupling of the excited state to a loss channel. As we have showed, this cannot be the cause of the major part of the effect because that should be an entire instantaneous effect and we see an ingrowth of the reduction, probably due to dielectric relaxation. Because also a loss of dipole strength in absorption is observed [3] we propose an electric field effect on the dipole strength possibly due to the mixing in of CT states which is enhanced due to the reaction field of the protein. The timescales on which we see the field effect enhanced are likely the timescales on which the LH2 protein moves at 77K. The protein reaction field has different components like an instantaneous component possibly due to charge redistribution, a component of hundreds of ps: rotation of charged and polar residues. This reorientation likely converts polarizability terms into permanent dipole moments thus increasing the field at the pigment cavity even more. The difference between the **ΔF/F** in steady state Stark fluorescence from what we report here and what is reported for the LH2 complex of *Rb. sphaeroides* in [5] could originate from the difference in polarizability which is equally great [3].
It's highly likely the processes reported here are a common feature of several bacterial photosynthetic complexes.

References

1. S. G. Boxer Biophysical techniques in Photosynthesis (1996) Kluwer Acad. Publ. pp177-189
2. Middendorf T. R., Mazzola L. T., Lao K. Steffen M. A. and Boxer S. G. (1993) Biochim. biophys. Acta 1144: pp 223-234
3. Beekman LMP, Frese RN, Fowler GJS, Picorel R, Cogdell RJ, Stokkum IHM van, Hunter CN, Grondelle R van (1997), *J. Phys. Chem. B 101*, p. 7293-7301
4. Frese RN, Oberheide U, Stokkum IHM van, Grondelle R van, Foidl M, Oelze J, Amerongen H van;*Photosynth. Res. 54*, 1997, p. 115-126.
5. Gottfried, D. S., Stocker, J. W. and Boxer, S. G. (1991) Biochimica et Biophysica Acta, 1059 pp. 63-75