S6-017

Time-resolved Fourier transform infrared difference spectroscopy for the study of A_1 reduction in intact photosystem I.

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Keywords: Time Resolved FTIR, Photosystem I, A1, Phylloquinone

Introduction

In photosystem one (PS I), the secondary electron acceptor, A_1 is a phylloquinone (PQ) species [Brettel., (1997); Golbeck and Bryant, (1991)]. There are two PQ's in PS I and recently the structural details of both PQ's and their binding site have been established using x-ray crystallography at 2.5 Å resolution [Jordan *et al.*, (2001)]: The two PQ's, are arranged symmetrically and both interact, via π-stacking, with the indole head groups of tryptophan residues (TrpA697 and TRP B677 in *S. elongatus*). In addition one carbonyl (C=O) of each PQ is hydrogen bonded to a backbone NH group (LeuA722 and LeuB706). At present it is unclear if one [Johnson *et al.*, (2000)] or both [Joliot and Joliot, (1999); Guergova-Kuras, *et al.*, (2001)] PQ's are involved in electron transfer (ET).

To test the structural predictions and address issues related to electron transfer directionality in PS I we have been using time resolved FTIR difference spectroscopy. Most FTIR studies performed to date on pigment-protein complexes use static photoaccumulation methods. It is extremely difficult to photoaccumulate A_1 and only a single (A_1^--A_1) FTIR difference spectrum has been reported [Hastings and Sivakumar, (2001)]. This spectrum could only be produced in PS I particles in which the terminal iron-sulfur clusters were deleted, and it is unclear if some of the observed infrared spectral features can be assigned to native PQ or were a result of structural modifications due to the harsh particle preparation procedure.

It is unlikely that static techniques will be useful in resolving ($P700^+A_1^-$-$P700A_1$) FTIR difference spectra simply because longer-lived components, such as $P700^+F_x^-$, will dominate the spectra. To produce (A_1^--A_1) FTIR DS in intact PS I particles it is therefore necessary to use time-resolved FTIR techniques. At 77 K, under repetitive illumination, $P700^+A_1^-$ recombines in ~45% of intact PS I particles with $\tau_{1/e}$ = 245 μs [fig. 1 and Schlodder *et al.*, (1998)]. With this in mind we have used FTIR difference spectroscopy with 5 μs time resolution to study intact PS I particles from *S. 6803* at 77 K.

Materials and Methods

Membrane fragments and purified monomeric PS I particles from the mutant psbDI/C/DII of *Synechocystis* sp. PCC 6803 (*S. 6803*) were prepared as described previously [Hastings *et al.*, (1995)]. Photo-accumulated ($P700^+$-P700) and ($P700^+A_1^-$-$P700A_1$) FTIR difference spectra were obtained as described previously [Hastings and Sivakumar, (2001); Hastings, (2001)]. Time resolved FTIR DS were also obtained as described previously [Hastings, (2001)]. For the present experiments a temperature controlled APD helium gas flow cryostat was coupled to the time resolved FTIR instrumentation. All spectra were collected at 4 cm^{-1} resolution.

Results

Fig. 2 shows the static (A_1^--A_1), ($P700^+A_1^-$-$P700A_1$), ($P700^+$-P700) FTIR DS obtained at RT using P700-A_1 particles [Hastings and Sivakumar, (2001)]. Clearly, photoaccumulation of A_1^- results in an intense negative band at 1680 cm^{-1}. Fig 3 shows the FTIR difference spectra obtained at multiple time delays after laser flash excitation of PS I particles at 77K. The photo-accumulated ($P700^+$-P700) FTIR DS at 77 K is also shown in fig. 3.

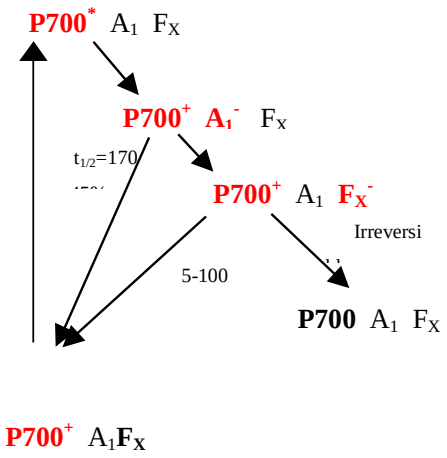

Figure 1: Kinetics in PS I at 77K, as outlined in Schlodder et al., (1998).

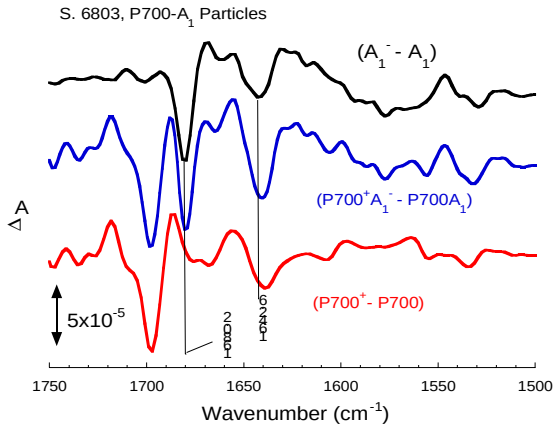

Figure 2: Static (A_1^--A_1) (*top*), ($P700^+A_1$ - $P700A_1$) (*middle*) and ($P700^+$-P700) FTIR DS in the 1750-1500 cm^{-1} spectral region, obtained using P700-A_1 PS I particles from *S. 6803*.

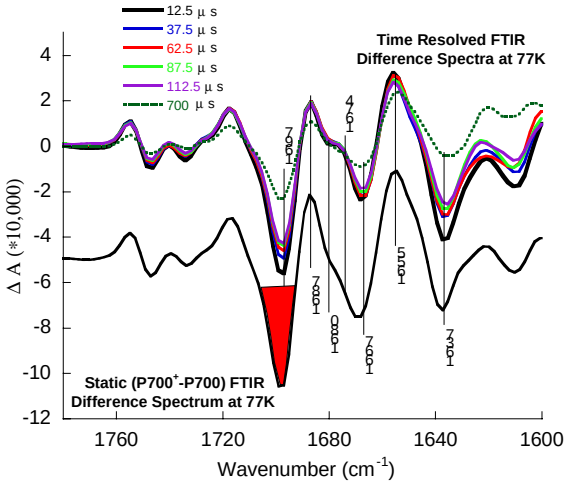

Figure 3: Static ($P700^+$-P700) and time-resolved ($P700^+A_1^-$-$P700A_1$) FTIR difference spectra at 77 K, obtained using intact PS I particles from *S. 6803* and a saturating flash repetition rate of 10 Hz.

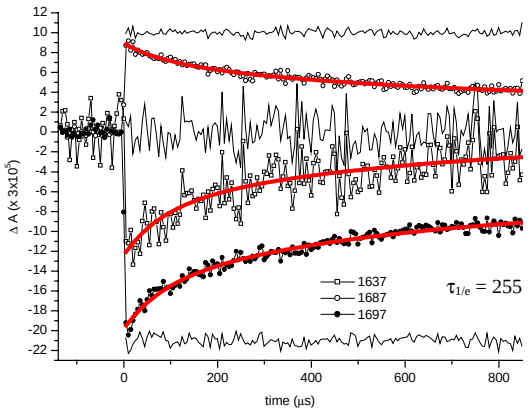

Figure 4: Kinetics of absorption changes observed at three IR frequencies following flash excitation at 10 Hz., obtained using intact PS I particles from *S. 6803*.

A baseline shift is observed in the 700 μs spectrum. Based upon the kinetic scheme in fig. 2 the 12.5-112.5 μs FTIR difference spectra are due predominantly to the $P700^+A_1^-$ radical pair state, while the 700 μs spectrum is due to $P700^+F_x^-$. Direct subtraction of early time spectra from late time spectra should therefore result in (A_1^- - A_1) FTIR difference spectra. The observed baseline drift considerably complicates this subtraction procedure. However,

the kinetics of the absorption changes can still be accurately monitored by considering the time change of the integrated area under a band, with appropriate baselines included (the shaded region of the static spectrum in fig. 3 shows such an area). This procedure is valid and accurate because the baseline drift is very broad.

Fig. 4 shows the time course of the change in the integrated areas at 1637, 1687 and 1697 cm^{-1}. Although precise frequencies are stated, each time point in the kinetics represents the area of an 8 cm^{-1} block, bounded by the time resolved spectra (collected every 5 μs) and centered around the stated frequency. Non-linear least square curve fitting procedures were applied simultaneously to the three time courses in fig. 4, assuming either a bi or mono-exponential decay process. The residual plots and fitted functions that result from the fitting procedure are also shown in fig. 4. The time constants obtained from a bi-exponential global analysis of the kinetics in fig. 4 were 82 and 556 μs. The quality of the bi-exponential fit was only slightly improved compared to a mono-exponential fit (as judged by residual plots and

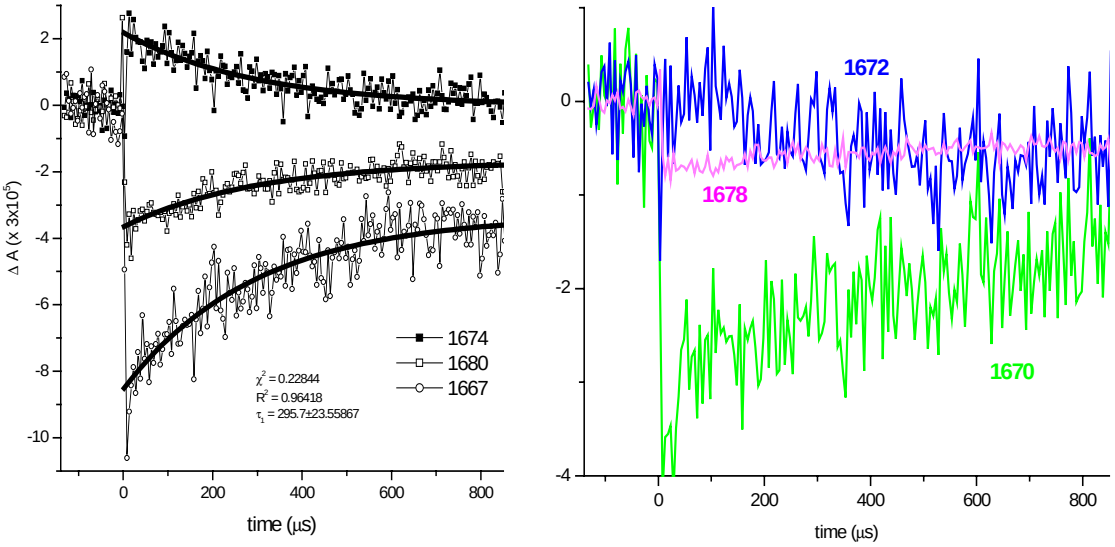

Figure 5: Kinetics of absorption changes observed at six IR frequencies following flash excitation at 10 Hz.

the reduced χ^2 parameter), which yielded a time constant of 255 μs. Within error, this latter time constant agrees exactly with that obtained from time resolved visible absorbance difference measurements [Schlodder *et al.*, (1998)].

Discussion

Based upon the kinetic scheme in fig. 2 the FTIR difference spectra within the first 100 μs should be due to the $P700^+A_1^-$ radical pair state. From the time resolved spectra in fig. 3, no intense negative band is observed near 1680 cm^{-1} (see also fig. 5). It is unlikely that the loss of the 1680 cm^{-1} band in the time resolved spectra is related to the temperature difference between the measurements. We therefore suggest that the 1680 cm^{-1} band in the static (A_1^--A_1) FTIR difference spectrum in fig. 2 is due to some structural alteration in the A_1 binding site induced by the harsh particle preparation procedure.

The initial bleaching at 1697 cm^{-1} has decayed by ~55% in 850 μs (fig. 4). In contrast the initial bleaching at 1637 cm^{-1} decays by ~78% in 850 μs (fig. 4). If the negative band at 1697

cm^{-1} is due only to P700 then $P700^+$ must decay in about 55% of the PS I particles with a lifetime of ~255 μs. We attribute this decay to $P700^+A_1^-$ recombination. In addition, the decay of the 1637 cm^{-1} kinetic would then indicate that A_1 reduction must give rise to a negative absorbance change at this frequency. This is consistent with the negative band observed near 1637 cm^{-1} in the (A_1^- -A_1) FTIR difference spectrum in fig. 2.

In the 1670-1680 cm^{-1} spectral region, the 12.5-112.5 μs time resolved spectra in fig. 3 are quite different in shape from the static spectrum, which shows significant negative absorption in this region. This indicates that A_1^- must contribute positive absorption changes in this spectral region, which is consistent with the static spectra in fig. 2. Fig. 5 shows the kinetics at several frequencies 1670-1680 cm^{-1} region. At 1674 cm^{-1} an initial positive absorption change decays almost to zero with a 295 μs time constant (within error, the same as in fig. 3). From the kinetic scheme in fig. 2 this indicates that all of the absorption change at 1674 cm^{-1} is due to (A_1^- -A_1), and that ($P700^+$-P700) does not contribute at 1674 cm^{-1}. These considerations are consistent with A_1^- having an absorption band in this region. However, the static ($P700^+$-P700) FTIR difference spectrum in fig. 3 is negative at 1674 cm^{-1}, and not zero. The discrepancy is likely a result of using an 8 cm^{-1} spectral region (1670-1678 cm^{-1} for the 1674 cm^{-1} kinetic) to calculate the kinetics. We are implementing methods to calculate kinetics at single frequencies without the use of integrated areas.

Conclusions

The (A_1^- -A_1) FTIR difference spectrum obtained using PS I particles that have been stripped of iron sulfur clusters displays spectral features that are difficult to reconcile with time resolved ($P700^+A_1^-$ -$P700A_1$) FTIR difference spectra at 77 K. These differences likely result from structural modifications that are induced in the PS I particles during removal of the iron sulfur clusters. We have obtained the first time resolved infrared absorption spectra associated with the decay of the $P700^+A_1^-$ radical pair state using intact photosystem I particles. By considering absorbance difference band profiles at several frequencies we were able to suggest spectral regions in which both A_1 and A_1^- absorb.

Acknowledgements

This work was supported by start-up funds, a quality improvement grant, a team-grant and a research initiation grant to GH from Georgia State University.

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