Time-resolved Fourier transform infrared difference spectroscopy for the study of A1 reduction in intact photosystem I.

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Introduction

In photosystem one (PS I), the secondary electron acceptor, A1 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 A1 and only a single (A1--A1) 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'+A1--P700A1) FTIR difference spectra simply because longer-lived components, such as P700'+Fx-, will dominate the spectra. To produce (A1--A1) FTIR DS in intact PS I particles it is therefore necessary to use time-resolved FTIR techniques. At 77 K, under repetitive illumination, P700'+A1- 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'+A1--P700A1) 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 \((A_1^- - A_1^-)\), \((P700^+ A_1^- - P700 A_1^-)\), \((P700^+ - P700^-)\) FTIR DS obtained at RT using P700-A1 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.

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
\text{cm}^{-1}. Although precise frequencies are stated, each time point in the kinetics represents the
area of an 8 \text{ cm}^{-1} block, bounded by the time resolved spectra (collected every 5 \text{ 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 \text{ 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.](image)

the reduced $\chi^2$ parameter), which yielded a time constant of 255 \text{ 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 \text{ 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 \text{ cm}^{-1} (see also fig. 5). It is unlikely that the loss
of the 1680 \text{ cm}^{-1} band in the time resolved spectra is related to the temperature difference
between the measurements. We therefore suggest that the 1680 \text{ 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 \text{ cm}^{-1} has decayed by $\sim$55% in 850 \text{ µs} (fig. 4). In contrast the
initial bleaching at 1637 \text{ cm}^{-1} decays by $\sim$78% in 850 \text{ 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 \(\sim 255 \mu s\). 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 \(\mu s\) 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 \(\mu s\) 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\(^{+}\) - P700\(_{A}\)) 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.

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References