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## **Transient EPR studies of Photosystem I containing non-native quinones**

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### **Introduction**

With the determination of the X-ray structure of Photosystem I (PS I) at a resolution of 2.5 Å (Jordan *et al* 2001), the details of the phylloquinone binding sites are now known. One of the two carbonyl oxygen atoms of each phylloquinone is H-bonded to the backbone nitrogen of Leu A722 or Leu B702 and the headgroup forms a close  $\pi$ -stack with Trp A697 or Trp B677. In addition, the phytyl tail serves as an anchor through hydrophobic interactions with the protein. However, this structural picture gives little information about the relative importance of these interactions and their functional significance remains unknown. Quinone exchange experiments provide a way of addressing this issue by allowing the effect on the binding associated with specific structural features to be investigated.

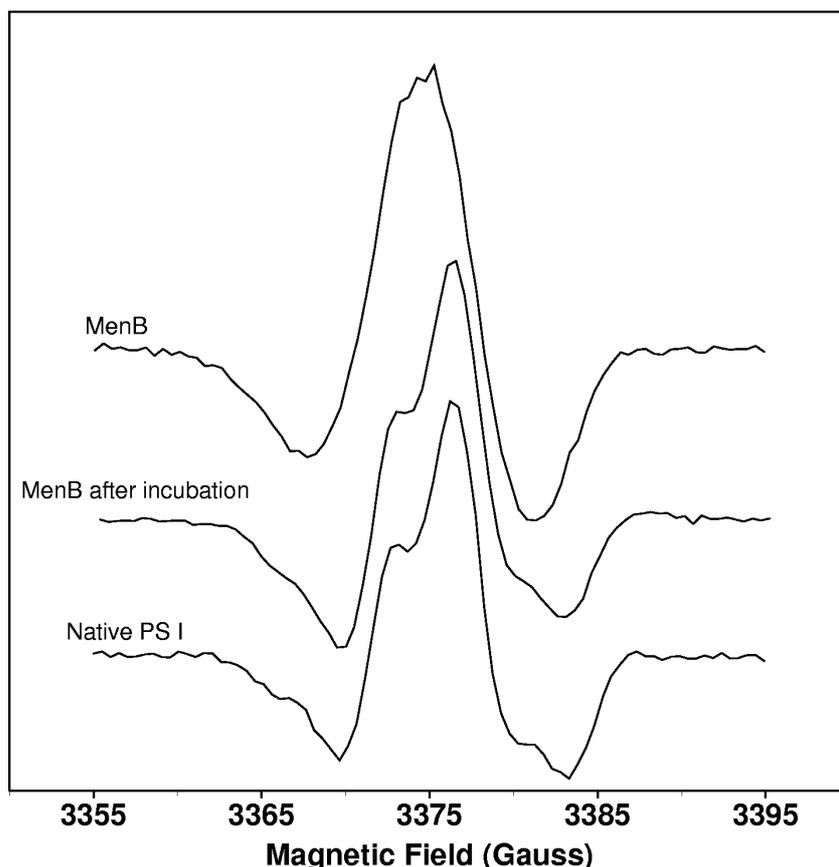
Recently, we have shown that the biosynthesis of phylloquinone can be inhibited by deletion of the *menA* and *menB* genes in *Synechocystis* PCC6803 (Johnson *et al* 2000) and in the absence of phylloquinone, PS I incorporates plastoquinone in A1 binding site (Zybailov *et al* 2000). Here we use transient EPR to study the displacement of plastoquinone when PS I from in the *menB* deletion mutant is incubated with various foreign quinones. The degree of displacement is determined by comparing the *menB* samples with solvent extracted native PS I incubated with the same quinones. This technique provides a convenient way of investigating the importance of individual interaction between the quinone and the protein.

### **Materials and methods**

PS I reaction centres were isolated from the wild type and *menB* mutant strains of *Synechocystis* 6803 as described in (Zybailov *et al* 2000). The wild type samples were extracted using hexane/methanol as described in (Sieckmann *et al* 1991). The extraction of the phylloquinone was monitored by the disappearance of the P<sup>+</sup>A1<sup>-</sup> EPR signal and appearance of <sup>3</sup>P<sub>700</sub> spectrum due to recombination from A0. The samples were then incubated with a 100-fold excess of a given quinone. 10 mM sodium ascorbate and 100 $\mu$ M DPIP were added as external redox mediators and the samples were frozen in the dark. X-band (9 GHz) and Q-band (35 GHz) transient EPR experiments were carried out using the set-ups described in (Zybailov *et al* 2000).

## Results

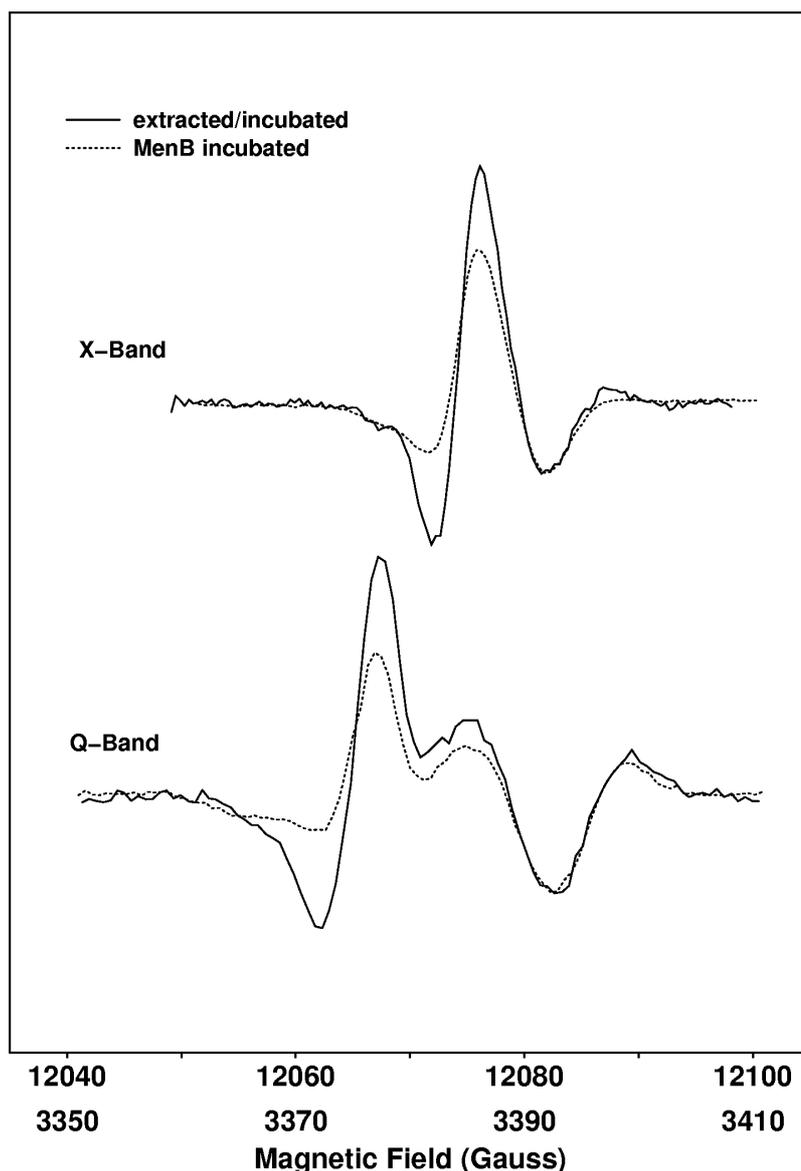
Fig. 1 shows a comparison of the X-band spectra of PS I from the menB mutant (top), the menB mutant following incubation with phylloquinone (middle) and the wild type strain (bottom). The spectra are spin polarized and are due to the state  $P^+Q^-$ . The differences between them arise from the different magnetic properties of phylloquinone and plastoquinone. From the fact that incubation of the menB sample with phylloquinone yields a



**Fig. 1** X-band transient EPR spectra of PS I at 150 K. The top spectrum is from the MenB mutant and the bottom is from wild type strain. The middle spectrum is the men B mutant sample after 15 min incubation with a 100 fold excess of phylloquinone at 5°C.

spectrum (middle spectrum) identical to that of native PS I (bottom spectrum), we conclude that phylloquinone completely displaces plastoquinone. This implies that the A1 binding site has a much higher binding affinity for phylloquinone than for plastoquinone. This difference in binding affinity is a logical consequence of the fact that PS I must bind phylloquinone in the presence of the plastoquinone pool associated with PS II. By investigating a series of quinones with structural features similar to those of plastoquinone or phylloquinone we can deduce which properties of the quinone are responsible for the difference in binding affinity.

Fig. 2 shows the MenB mutant incubated with anthraquinone (dotted curves). For comparison, spectra of solvent extracted PS I also incubated with anthraquinone are shown (solid curves). As can be seen the intensity on the low field side of the side of the spectra is considerably greater for the solvent extracted/incubated sample. The intensity in the quinone region is determined largely by the inhomogeneous linewidth arising from hyperfine couplings.



**Fig. 2** X-band (top) and Q-band (bottom) transient EPR spectra of quinone exchanged PS I containing anthraquinone.  $T=150$  K. The solid curves are of solvent extracted PS I from wild type *Synechocystis*, the dotted curves are of PS I from the *menB* deletion mutant. In both cases the PS I was incubated with an excess of anthraquinone.

Because the couplings in anthraquinone are weaker than those in plastoquinone we expect an increase in intensity if anthraquinone displaces plastoquinone. The lower intensity for the *MenB* sample (dotted curves) compared to the extracted sample, which contains only anthraquinone (solid curves), indicates that the displacement of the plastoquinone by anthraquinone is not complete. Taking into account the fact that there is an excess of anthraquinone in the sample, the results in Fig. 2 suggest that the binding affinity of anthraquinone is weaker than that of plastoquinone.

## Discussion

We have studied a series of quinones of various structures in this way and our initial results indicate that the nature of the quinone headgroup plays a much more important role than the phytol tail. Thus, for example, hydrolysis of the double bond in the phytol tail of phylloquinone has little effect on its binding affinity relative to plastoquinone despite the change in polarity and conformation of the side chain. In contrast, replacement of the naphthoquinone headgroup in phylloquinone with benzoquinone derivatives dramatically lowers the degree of displacement of plastoquinone. Moreover, naphthoquinone and naphthoquinone derivatives with a variety of small side chains all effectively displace plastoquinone. These findings suggest that the A1 binding site is tailored to preferentially bind naphthoquinone derivatives over benzoquinone derivatives and that this difference in binding affinity is used to assure that phylloquinone and not the more abundant plastoquinone occupies the binding site in PS I. How this preferential binding affinity is achieved is still an open question. However, it is clear that the close  $\pi$ -stacking to Trp A697 and Trp B677 is the protein-cofactor interaction, which is most likely to differ between benzoquinone and naphthoquinone derivatives and may be responsible for the difference in binding strength. This is also in contrast to type II reaction centres in which the quinone binding is dominated by a strong H-bond to histidine and a variety of both benzoquinone and naphthoquinone derivatives such as plastoquinone, ubiquinone and menaquinone are bound.

## Acknowledgments

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