# Regulation of photosynthesis -Cytochrome b6f in redox regulation -Two novel proteases acting on an N-terminal peptide of LHCII

Ström, Jörgen

2005

# LOW-TEMPERATURE ABSORPTION AND MAGNETIC CIRCULAR DICHROISM OF THE FOUR HAEMS OF THE CHLOROPLAST CYTOCHROME $b_6f$ COMPLEX

Sindra Peterson Årsköld[1], Jörgen Ström[2], John F. Allen[2], Elmars Krausz[3]. [1]Department of Biochemistry and [2]Department of Plant Biochemistry, Center for Chemistry and Chemical Engineering, Lund University, PO Box 124, S-22100 Lund, Sweden. [3]Research School of Chemistry, Australian National University, Canberra ACT 0200, Australia

***Keywords:*** Cytochrome $b_6f$, Cytochrome $c_i$, MCD, absorption, spectroscopy

## INTRODUCTION

The cytochrome (cyt) $b_6f$ complex is situated in the thylakoid membrane, where it mediates linear electron transfer between photosystems II and I and performs cyclic electron transfer. Both processes result in proton translocation across the membrane, creating an electrochemical gradient for ATP synthesis. Homologous to the mitochondrial cytochrome $bc_1$ complex, cyt $b_6f$ incorporates cytochrome $f$ (a $c$-type cytochrome) and an $Fe_2S_2$ protein, constituting the linear electron pathway from hydroplastoquinone to plastocyanin, and cytochrome $b_6$, containing the haem groups $b_H$ and $b_L$ involved in cyclic electron transfer. Unlike the mitochondrial complex, the $b_6f$ complex also contains a chlorophyll (chl) $a$, a β-carotene (car), and a fourth haem, haem $c_i$ (reviewed by Allen (2004)). The presence of haem $c_i$ at the stromal quinone binding site came as a surprise when the X-ray crystal structure was solved last year, for cyt $b_6f$ purified from the unicellular green alga *Chlamydomonas reinhardtii* (Stroebel et al 2003) and from the thermophilic cyanobacterium *Mastigocladus laminosus* (Kurisu et al 2003). The Fe of haem $c_i$ is penta-coordinate, with water (or hydroxide) the only axial ligand. This indicates a high-spin configuration of the haem, which would explain why it has not been predicted from previous spectroscopic studies.

We used low-temperature, high-precision optical polarization spectroscopies to investigate the chromophores of the cyt $b_6f$ complex. In particular, magnetic circular dichroism (MCD) was used to monitor the four haem groups in reducing and oxidizing environments. As the number of haem groups is unambiguously known from the crystal structure, and MCD shows quantitative signals from cytochromes poised in any redox and spin state, this approach promises further rigorous results.

## MATERIALS AND METHODS

Cyt $b_6f$ was isolated from 12-day-old pea leaves, following Hurt and Hauska (1981), with the dialysis step replaced by a Phenyl (High Sub) column, followed by concentration of the fractions of interest using Microsept 10 K Omega.

For the reduced samples, dithionite was added in excess and left to incubate in the dark, on ice, for 5–10 min. Then, glycerol was added to give a final concentration of 60% (v/v), and the mixture was rapidly inserted into a quartz-windowed cell and glassed into He($l$). For the oxidized samples, periodate replaced dithionite.

The spectrometer (described by Peterson Årsköld et al (2003)) allows simultaneous detection of absorption and (M)CD, making analysis of (M)CD spectra in terms of the corresponding absorption spectra exact and reliable. The MCD traces presented are the differences between spectra recorded at +5T and −5T.

## RESULTS AND DISCUSSION

**1.7-K Absorption and MCD Spectra of cyt $b_6f$.** In Fig. 1A, the 1.7-K absorption spectrum of cyt $b_6f$ is displayed, and the origin of each spectral contribution specified. Chl $a$ dominates the spectrum, with a strong Soret band at 420 nm and the $Q_y$ transition at 670 nm. The vibrational side-bands of the $Q_y$ peak and the $Q_x$ transition are present in the 550–650 nm region. These features identify the pigment as a regular chl $a$. The β-car absorption is also visible, at 488 nm.

The peaks at 548 and 551.5 nm have been assigned to the $Q_x$ and $Q_y$ transitions of the reduced form of cyt $f$ (Schoepp et al 2000). The vibrational side-bands are discernible in the 500–550 nm region. The presence of these features establishes that in our preparation, cyt $f$ is reduced to a significant extent, while the other haem groups are oxidized. The absorption features of oxidized, low-spin haem are spread over 400–600 nm, but are too weak to stand out under the chl $a$ Soret band. However, these features are accompanied by strong MCD signals, clearly visible in Fig. 1B (solid line). Despite its strong aborption, chl does not contribute to the MCD in this region.

Low-spin oxidized haem gives rise to a strong MCD C-term at 420 nm, along with a series of weaker features reaching above 600 nm. C-terms are paramagnetic in origin, and are distinguished by strong temperature dependence. The 50-K MCD (Fig. 1B, dashed line) identifies the cyt C-term from other absorptions. The derivative-shaped MCD A-term associated with reduced, low-spin haem around 550 nm is unaffected by temperature, as is the weak chl B-term at 670 nm. The A-term of reduced cyt $f$ is shown in detail in Fig. 2 (thick, solid lines). The positive and negative components of the A-term establish the exact wavelengths of the Q transitions, and the vibrational side-bands are clearer in the MCD than in the absorption.

**MCD Spectra of Fully Reduced and Fully Oxidized cyt $b_6f$.** *Fully reduced cyt $b_6f$.* Figure 1C shows the 1.7-K absorption (thick, solid line) and MCD (thin solid and dotted lines) of cyt $b_6f$ reduced by dithionite. Under these conditions, there is no sign of the low-spin, oxidized cyt C-term. At 430 nm however, a temperature-dependent MCD signal characteristic of high-spin, ferric haem has appeared. The thin, solid lines in Fig. 2 show the green spectral region in detail. Features from reduced cyt $f$ are similar to those in the untreated samples. Additional peaks are seen at 556.6 and 561.8 nm which can be assigned to a superposition of reduced haems $b_H$ and $b_L$ (Schoepp et al 2000). The MCD A-term accompanying these peaks is broad and asymmetric, supporting the conclusion that this is a composite signal.

*Fully oxidized cyt $b_6f$.* The dashed line in Fig. 2B shows the MCD of cyt $b_6f$ under oxidizing conditions. All signs of reduced haem are gone, while a strong C-term from oxidized haem is present. There are no discernible absorption features in this region (not shown).

 

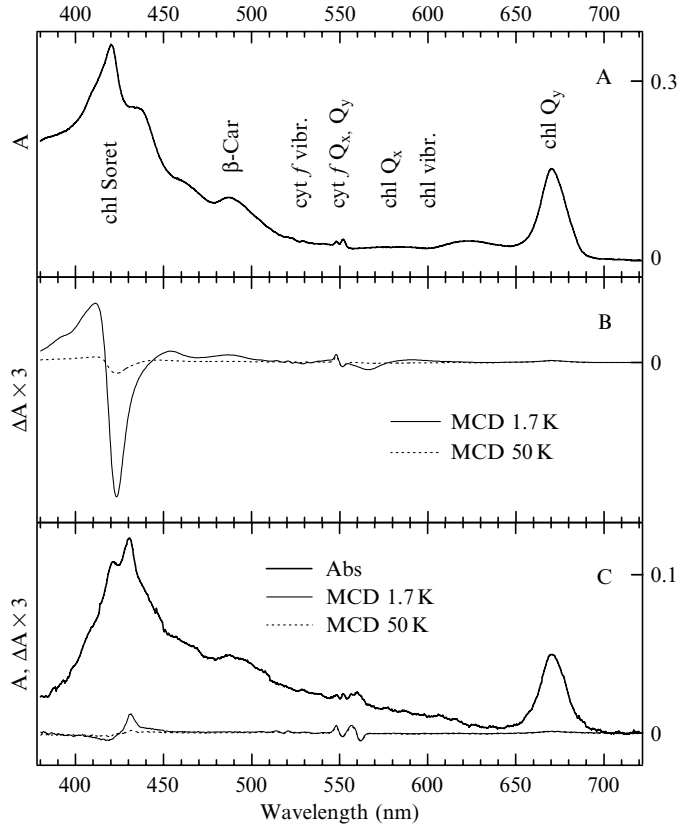


**Figure 1:** (A) 1.7-K absorption spectrum of the cyt $b_6f$ complex. (B) The corresponding MCD at 1.7 K (solid) and 50 K (dashed). (C) 1.7-K absorption, 1.7-K and 50-K MCD of fully reduced cyt $b_6f$. The data are plotted to equal chl $Q_y$ intensities in all samples, for quantitative comparison. The MCD data are magnified 3 times compared with the absorption.

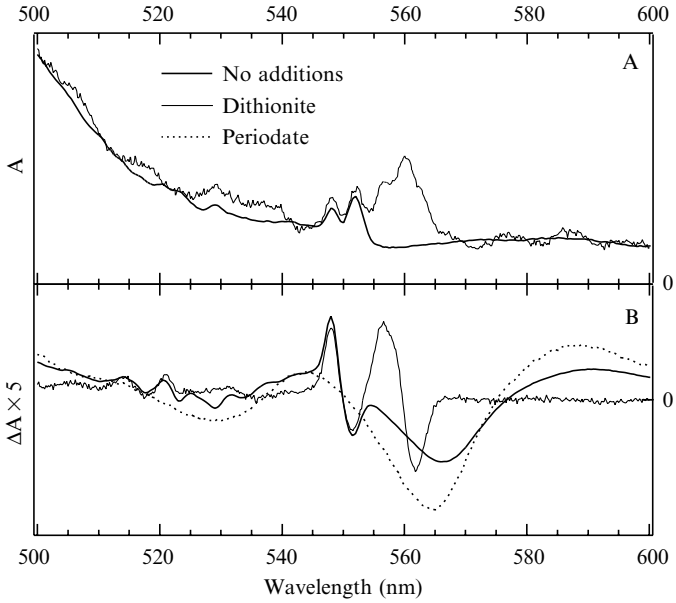


**Figure 2:** 1.7-K absorption (A) and MCD (B) of cyt $b_6f$ without reactants, with dithionite (all cyt reduced), and with periodate (all cyt oxidized; MCD only). The data are plotted to equal chl $Q_y$ intensities. The MCD data are magnified 5 times.

**Quantitative Analysis of the Haem Content of each cyt $b_6f$ Sample.** Cytochromes give rise to clearly distinguishable and quantitative MCD signals in the ferrous low-spin, ferrous high-spin, and ferric low-spin states. Ferric high-spin haem also gives rise to a distinctive MCD signature, but this signal is too small to be detected among other haem signals. The various forms of cytochrome MCD have been comprehensively reviewed by Cheesman et al (1991).

In the periodate-treated sample (Fig. 2B, dashed line), 100% of the low-spin population is oxidized, as seen by the absence of A-terms around 550 nm and the presence of a strong C-term in the same region. Using this C-term to quantify the C-term present in the non-treated sample (thick, solid line), we find that 60% of the low-spin population is oxidized in this sample. From the location of the single A-term in the non-treated sample, we conclude that the remaining 40% of low-spin haem is reduced cyt *f*.

In the dithionite-treated sample, there is no oxidized low-spin haem. The cyt *f* A-term at 550 nm was not increased by the treatment, indicating that it was fully reduced in the untreated sample. The magnitude of the cyt *b* absorption compared to cyt *f* indicates that cyt $b_H$ and $b_L$ are fully reduced (Schoepp et al 2000) under these conditions.

In the present report, we demonstrate the presence of high-spin ferrous haem in cyt $b_6f$ under reducing conditions (Fig. 1C). We assign this to cyt $c_i$. Consistent quantification of the ferric cyt MCD signals requires that ferric cyt $c_i$ is high-spin as well. Comparing the high-spin, reduced cyt MCD signal at 430 nm (Fig. 1C) to the literature (Cheesman et al 1991), it appears about 50% as strong as expected. This low intensity could mean that only half of the cyt $c_i$ population is reduced by dithionite, leaving half in the (not discernible) ferric high-spin state. This would require quite a low redox potential of cyt $c_i$, which is perhaps less likely, seeing that it is positioned next to the inner quinone binding site and is therefore likely to undergo frequent redox chemistry. Alternatively, the nature of cyt $c_i$ could be such that the ferrous MCD signal is inherently weak. This would be expected if the spin-orbit coupling of the excited states is low (Cheesman et al 1991).

## CONCLUSIONS

Our MCD data from the cyt $b_6f$ complex under different conditions are consistent with cyt $c_i$ being in a high-spin configuration and cyt *f*, $b_H$ and $b_L$ in low-spin configurations, irrespective of redox state.

In the untreated sample, cyt *f* is fully reduced (low-spin), cyt $b_L$ and cyt $b_H$ are fully oxidized (low-spin), as is cyt $c_i$ (high-spin). Periodate fully oxidizes cyt *f*, cyt $b_H$ and cyt $b_L$ (low-spin), as well as cyt $c_i$ (high-spin). Dithionite reduces cyt *f*, cyt $b_H$ and cyt $b_L$ fully (low-spin), and reduces cyt $c_i$ (high-spin) to 50% or more.

We believe cyt $c_i$ to be fully reduced by dithionite, but with inherently weak MCD, indicating weak spin-orbit coupling between the excited states.

## ACKNOWLEDGMENTS

SPÅ acknowledges grants from *Kungliga Fysiografiska Sällskapet i Lund* and *Stiftelsen Landshövding Per Westlings Minnesfond*. We thank Derek Bendall for suggestions on sample preparation.

## REFERENCES


Allen, J. F. (2004) *Trends in Plant Sci.* 9: 130–137.

Cheesman, M. R., Greenwood, C. & Thomson, A. J. (1991) *Adv. Inorg. Chem.* 36: 201–255.

Hurt, E. & Hauska, G. (1981) *Eur. J. Biochem.* 117: 591–599.

Kurisu, G., Zhang, H., Smith, J. L. & Cramer, W. A. (2003) *Science* 302: 1009–1014.

Peterson Årsköld, S., Masters, V. M., Prince, B. J., Smith, P. J., Pace, R. J. & Krausz, E. (2003) *J. Am. Chem. Soc.* 125: 13063–13074.

Schoepp, B., Chabaud, E., Breyton, C., Verméglio, A. & Popot, J.-L. (2000) *J. Biol. Chem.* 275: 5275–5283.

Stroebel, D., Choqet, Y., Popot, J.-L. & Picot, D. (2003) *Nature* 426: 413–418.


 