

implicates a bifurcated H-bond to both the inhibitor's carbonyl oxygen and the thiazole sulfur. The nitrile nitrogen is H-bonding Ser39 of the anchor subunit C. Additional van der Waals contacts between the aromatic rings of thiapronil and putative Q site amino acid residues are discussed.

## References

- [1] G. Cecchini, *Annu. Rev. Biochem.* 72 (2003) 77–109.
- [2] L.S. Huang, et al., *J. Biol. Chem.* 281 (2006) 5965–5972.
- [3] R. Horsefield, et al., *J. Biol. Chem.* 281 (2006) 7309–7316.
- [4] F. Sun, et al., *Cell* 121 (2005) 1043–1050.

doi:10.1016/j.bbabbio.2010.04.074

## 1P.27 Photosynthesis with simplified cytochrome *b<sub>6</sub>f* complexes: Are all hemes required?

Alizée Malnoë<sup>1</sup>, Jacqueline Girard-Bascou<sup>1</sup>, Frauke Baymann<sup>2</sup>, Jean Alric<sup>1</sup>, Fabrice Rappaport<sup>1</sup>, Francis-André Wollman<sup>1</sup>, Catherine de Vitry<sup>1</sup>

<sup>1</sup>CNRS, UMR 7141, France

<sup>2</sup>CNRS, UPR 9036, France

E-mail: alizee.malnoe@ibpc.fr

Cytochrome *bc<sub>1</sub>* and *b<sub>6</sub>f* complexes are key players in bioenergetic electron transfer chains. Their quinol oxydoreductase activity participates to the formation of the proton motive force through the Q-cycle. Structural data (Stroebel *et al.*, 2003, *Nature* **426**: 413–418) showed that *b<sub>6</sub>f* differs from *bc<sub>1</sub>* by three additional cofactors: one  $\beta$ -carotene, one chlorophyll *a* and a singular heme, named *c<sub>i</sub>*, located in the quinone reduction site. The CCB maturation pathway specifically responsible for the delivery of *c<sub>i</sub>* and its covalent binding has been described recently (Kuras *et al.*, 2007, *Proc. Natl. Acad. Sci. USA* **104**: 9906–9910). A *ccb* mutant shows low accumulation level of functional *b<sub>6</sub>f* complex and, hence, cannot grow photosynthetically (Saint-Marcoux *et al.*, 2009, *J. Cell Biol.* **185**: 1195–1207). This inability to grow under phototrophic conditions grounded a screen for suppressor mutations allowing accumulation level of functionally active *b<sub>6</sub>f* complex compatible with photosynthetic growth, yet still lacking the *c<sub>i</sub>* heme. The genetic analysis of the thereby rescued mutants showed that the suppressor mutation is nuclear, monogenic and affects a chloroplast protease. Although phototrophic, this mutant is highly photosensitive in the presence of oxygen. Spectroscopic study of the purified *b<sub>6</sub>f* complex confirmed the absence of *c<sub>i</sub>*. *In vivo* functional analysis showed that the turnover of the variant *b<sub>6</sub>f* complex is not electrogenic showing that the quinone reduction site is inactive. Yet, we could observe the usual oxidant induced reduction of a *b* heme and this reduction phase was similar to the WT one, thus showing that the quinone oxidation site is not impaired. Altogether these findings show that *b<sub>i</sub>*, the *b* heme of the quinone reduction site, does not participate to the turnover of the complex. Consistent with this, redox titration evidenced a strong down-shift of the midpoint potential of one of the two *b* hemes and we assigned the more negative midpoint potential to the *b<sub>i</sub>* heme, excluding it, on thermodynamic ground, from the functional field. The combination of the suppressor mutation to a mutant bearing a substitution of the His202 axial ligand of the *b<sub>i</sub>* heme, allowed us to construct a mutant lacking the *b<sub>i</sub>* heme but still accumulating a high level of *b<sub>6</sub>f* complex. This variant grows under photosynthetic conditions providing the final demonstration that the turnover of this minimal *b<sub>6</sub>f* complex sustains an electron transfer flux compatible with photosynthetic growth despite its inactive Q-cycle.

doi:10.1016/j.bbabbio.2010.04.075

## 1P.28 Synthesis of cardiolipin analogues bearing a biophysical probe at any position of the four acyl chains

Masato Abe, Shunya Koubori, Hideto Miyoshi

Division of Applied Life Sciences, Graduate School of Agriculture, Kyoto University, Japan

E-mail: abe@kais.kyoto-u.ac.jp

Cardiolipin (CL), a negatively charged phospholipid bearing four fatty acid chains, is a major phospholipid found in mammalian mitochondria (up to 20–25%) with a multitude of biological functions. For instance, CL is responsible for regulation of the activity of several mitochondrial enzymes involved in ATP biosynthesis, though the precise molecular mechanism of regulation remains to be elucidated. This is primarily because CL analogues used in the previous biochemical studies are limited to natural and/or a few commercially available CL analogues; in the former, the chain moiety is a mixture of various fatty acids, and in the latter, the chemical variation of fatty acid chains is very poor. Therefore, to explore in detail the molecular mechanisms of both the formation of the *cyt c*-CL complex and the induction of peroxidase activity of *cyt c*, biochemical studies using structurally variable CL analogues are needed. Several different procedures for the synthesis of CL have been reported. Previous studies however were not necessarily concerned with generating structurally diverse CL analogues. For example, some procedures for the synthesis of CL bearing only saturated fatty acid chains are not suitable for the synthesis of CL containing linoleic acid(s) (C18:2), which is a major fatty acid of natural CL in mammalian mitochondria, because the *cis*-1,4-diene structure in linoleic acid is remarkably degradable under the conditions. In addition, some methods are not feasible for the routine preparation of large quantities owing to use of highly unstable intermediates or expensive reagents. The phosphoramidite approach, widely exploited in oligonucleotide chemistry, described by Ahmad *et al.* is an excellent way to obtain large quantities of CL analogues in high yields [1]. Unfortunately, they did not use linoleic acid as the acyl chain(s), and their procedure do not give asymmetrically substituted CL analogues. We now developed a concise procedure for the synthesis of CL using phosphoramidite chemistry, which produces diverse CL analogues bearing linoleic acid(s) at any position of the four acyl chains on a gram scale. This approach also allows for the production of CL containing a biophysical probe (nitroxide spin-label, fluorescent label, etc.) in one of the four chains.

## Reference

- [1] U.M. Krishna, *Tetrahedron Lett.* 45 (2004) 2077–2079.

doi:10.1016/j.bbabbio.2010.04.076

## 1P.29 Surface enhanced infrared absorption spectroscopy (SEIRAS) of complex I and QFR from *Escherichia coli*

Frederic Melin<sup>1</sup>, Sebastien Krieger<sup>1</sup>, Laurent Fremont<sup>2</sup>, Thorsten Friedrich<sup>3</sup>, Elena Maklashina<sup>4</sup>, Gary Cecchini<sup>4</sup>, Petra Hellwig<sup>1</sup>

<sup>1</sup>Institut de Chimie UMR 7177, Université de Strasbourg, France

<sup>2</sup>Laboratoire de Chimie de Coordination, Université Paul Sabatier, Toulouse, France

<sup>3</sup>Institut für Organische Chemie und Biochemie, Albert-Ludwigs-Universität, Freiburg, Germany

<sup>4</sup>Molecular Biology(151-S), Veterans Affairs Medical Center, San Francisco, USA

E-mail: hellwig@chimie.u-strasbg.fr

Infrared spectroscopy was established as a very valuable method for the study of the structure and dynamics of enzymes. The mid-IR domain (4000–500 cm<sup>-1</sup>) gives information on the secondary