ESRF	**Experiment title:** Structural studies on the chloroplast F_1F_0-ATPase	**Experiment number**: MX-23
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Report:

ATP synthases found in the energy transducing membranes of bacteria, mitochondria and chloroplasts catalyse ATP synthesis and ATP hydrolysis coupled with a transmembrane proton transport. The enzymes are multi-subunit complexes composed of an extra-membranous catalytic F_1 domain and a membrane intrinsic F_0 domain.

The chloroplast ATP synthase located in the stroma lamellae of the thylakoid membranes shares many structural and functional characteristics with the homologous mitochondrial and bacterial ATPases, but is unique in enzyme activation and in the interaction with specific energy transfer inhibitors. In contrast to ATPases from other species and organelle the activity of the chloroplast enzyme is strongly controlled by the transmembrane proton gradient and the redox state of the γ-subunit in the membrane extrinsic F_1 domain [reviewed in 1]. Catalytic and activating proton transfer reactions of the chloroplast ATPase were discriminated by specific inhibitors and spectroscopic probes [2]. However, the location of the protonation sites that correspond to these proton transfer reactions is not resolved yet.

The structure of the chloroplast $\alpha_3\beta_3$ core compolex and of a co-complex containing the chloroplast specific phytopathogenic inhibitor tentoxin have been solved in the past by our group [3-4]. In order to obtain further structural information on the regulatory γ-subunit and to resolve the binding site and the mechanism of another specific CF_1-inhibitor we have crystallized the soluble chloroplast F_1-domain in the presence of Ajmaline, a dihydroindole alkaloid, which was shown to specifically

inhibit energy transfer and block photophosphorylation in spinach chloroplast. For arresting of the γ-subunit in the $\alpha_3\beta_3$-core the non-chloroplast specific inhibitor bathophenanthroline was used.

Data sets of CF_1-Ajmaline and CF_1-Bathophenanthroline were collected during 5 shifts on ID14-2. Data were processed by DENZO/SCALEPACK on site. All crystals showed mosaicities of almost 1.0, belong to space group *R32* and have unit cell parameter similar to the native CF_1-complex (a = 147 Å, b = 147 Å, c = 385 Å).

Data set	Resolution [Å]	R-merge [%]	Completeness [%]	I/σI (outer shell)
CF_1-Ajmaline	4.2	7.2	68.5	3.6
CF_1-BPA	3.5	14.6	90.0	2.1

In addition we have collected diffraction data on crystals obtained with the purified CF_1CF_o-holoenzyme. During the 5 shifts on ID14-2 we have collected 1 data set which was processed by MOSFLM/SCALA.

Data set	Resolution [Å]	R-merge [%]	Completeness [%]	I/σI (outer shell)
CF_1F_o	3.8	5.5	81.0	2.3

Refinement of these data is still in progress.

References

[1] Groth, G. and Strotmann, H. 1999, Physiologia Plantarum 106, 142-148.

[2] Groth, G., and Junge, W. 1995, FEBS Lett. 358, 142-144.

[3] Groth, G. and Pohl, E. (2001) *J. Biol. Chem.* 276, 1345-1352.

[4] Groth, G. (2002) *Proc. Natl. Acad. Sci. USA*, 99, 3464-3468.