ESRF	**Experiment title:** **Structural dynamics of ATP-synthase and its catalytic F1-fragment during the reaction cycle of ATP hydrolysis**	**Experiment number**: LS722
Beamline: ID02 / BL4	**Date of experiment**: from: 3.9.1997 to: 6.9.1997	**Date of report**: 28.1.1998
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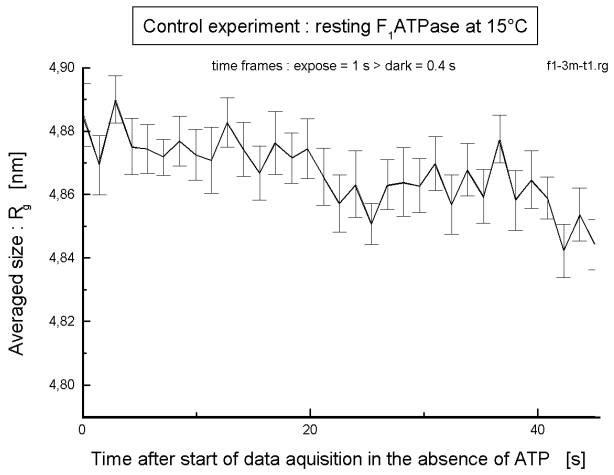
Report:

Conclusion:

0) The experimental session LS722 was succesfull in investigation of working F1ATPase and ATP-synthase with the full flux of the ID02 beamline using a CCD camera as proposed in the application:
1) The enzymes F_1ATPase and complete ATP-synthase withstand the full radiation (10^{13} ph/s) for a time sufficient for estimation of a structural film (>40 s) in the presence of a radiacal scavenger and de-gasing.
2) Both enzymes can be kinetically synchronized even under full X-ray beam by an ATP concentration jump.
3) During the reaction cycle of F_1ATPase significant size changes were observed (ΔR_g = 2 Å). First experiments with ATP-synthase detected those, but with weaker statistics (object of new experiment 6/98).
4) The size pulses changed their kinetics with the temperature parallel to the enzyme reaction kinetics.
5) The structural film showed three short expansion pulses, and a terminal short shrink pulse during the reaction cycle. The occurence and time structure was the very first time detected by our experiments.
6) The synchronization was stable for at least three subsequent cycles (1 ATP each) as observed earlier [4]. Possibly 3 subsequent cycles are part of a supercycle of the 3 catalytic centers present in the enzyme.
7) Obviously the large subunits (α,β) of the enzyme are rearranged in a cyclic manner during the reaction cycle. According to model calculations (FVM cube models [6]) this structure dynamics is not produced by the gamma-subunit rotation observed by fluorescence (the effect of this central mass is much weaker). Probably there is a hirarchy of three movements inside ATPase:
   $F_o$part-movement $\Leftrightarrow$ gamma rotation $\Leftrightarrow$ F_1ATPase subunit movement.
8) The unexpectedly short duration of the structure pulses (<400 ms; 3% cycle) produced a problem with the read-out blind phases of the CCD-camera. This effect is known as "aliasing" and produces an extra-error. The "aliasing" effect is enhanced by small variations of the start lag time (50-100 ms) during the activation of the enzyme by the substrate concentration jump.



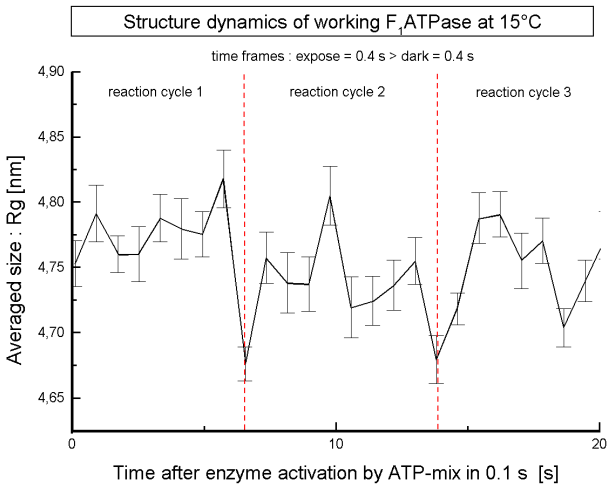


Fig.1: No changes occur in resting enzyme. Fig.2: Structure dynamics working of ATPase

For the investigation of working proteins we have developped a helium-jet cooled sample environment and a stopped-flow device. With a CCD camera the full flux of ID02 was used. As shown in the LS722b /717 report, the enzymes were >40s stable with radical scavengers. By a substrate concentration jump the reaction cycle of ATP hydrolysis of F1ATPase and ATP-synthase from Micrococcus luteus can be started and synchronized. The structure was shown to change transiently earlier qualitatively at DESY [4]. As shown in Fig.1 (control experiment) and Fig.2 (working F_1ATPase) both events necessary for taking a structural film occur also at 1000 fold increased flux at ESRF-ID02 (10^{13} ph/s at 0.2 * 0.8 mm). At ESRF we observed novel structural features of working F_1ATPase and ATP-synthase [5]:

- In the control experiment (F_1ATPase, no ATP) no changes were observed (no artefact).
- With working enzyme (F_1ATPase + CaATP) a series of 3 short expansion pulses occurs. The reaction cycle is terminated by a typical shrink pulse (by this the cycle is easyly located). The shrink pulse was also visible in first experiments with complete ATP-synthase.
- In contrast to [4] the results were obtained with "single shots". The structural changes appear as unexpectedly short pulses (< 400 ms at 13 s cycle time, yielding an aliasing error)
- The time scale varied with the same law as the enzymatics with the temperature (± 20%).
- The structure dynamics was visible for at least 3 subsequent reaction cycles, as observed earlier [4] and by enzyme kinetics (1 ATP/molecule, but the enzmye contains 3 cooperatively coupeled enzymatic centers [2]). Thus possibly 3 subsequent "sub-cycles" form a "supercycle" of ATPase.
- The location and more precise estimation of the short time events is important for the interpretation of the results: Modeling (cube method FVM [6]) showed, that the observed structural pulses are not caused by the rotation [1,3] of the central gamma-subunit inside the hollow F1ATPase complex. Obviously the large subunits 3*(α,β) are rearranged in a cyclic manner inside the enzyme during the reaction cycle. Probably there is a hirarchy of three movements inside ATPase. In contrast to [1,3] we observed the last sequence part:

$F_o$part-movement ⇔ gamma rotation ⇔ F_1ATPase subunit movement

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2. Abrahams, J.P.; Leslie, A.G.W.; Lutter, R.; Walker, J.E. (1994) Nature 370, 621-628 (crystallography)
3. Noji, H.; Yasuda, R.; Yoshida, M.; Kinosita, K. (1997) Nature 386, 299-302 (fluorescence study)
4. Neidhardt, A., Nawroth, T.; Hütsch, M.; Dose, K. (1991) FEBS Lett. 280, 179-182
5. Nawroth, T. (1997) extra-talk at ESRF user meeting and 2 late posters at SR50 conference
6. Nawroth, T. (1989) Physica B 156, 493-495 (improved PC-version available for modelling dynamics)