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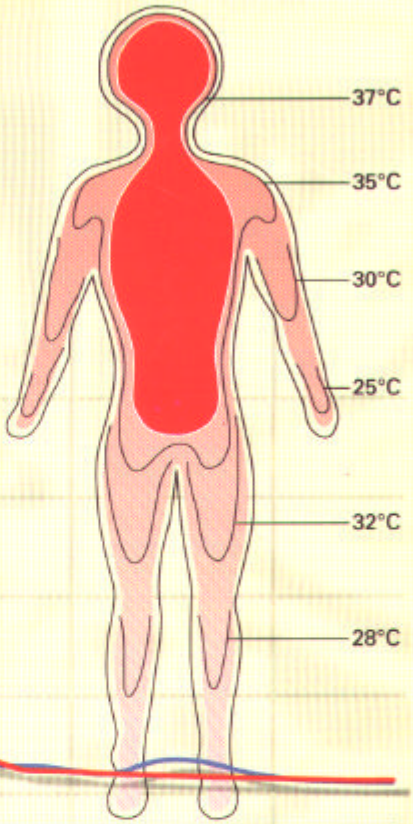

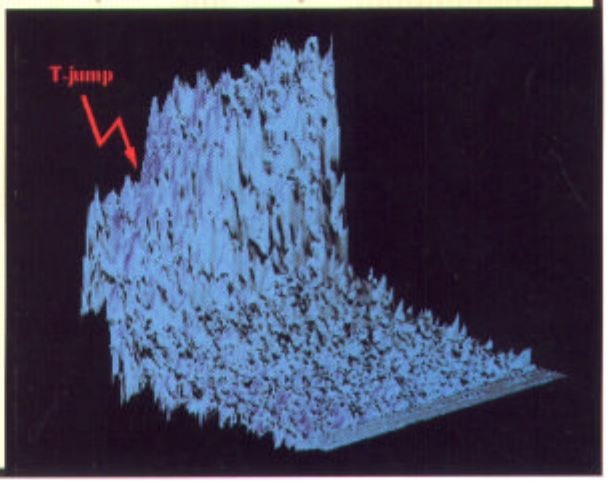

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AUSTRIAN SAXS BEAMLINE AT elettra

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STRUCTURE DYNAMICS OF F_1ATPASE DURING AZIDE-INHIBITION OBSERVED BY TIME-RESOLVED X-RAY SMALL ANGLE SCATTERING (TR-SAXS)

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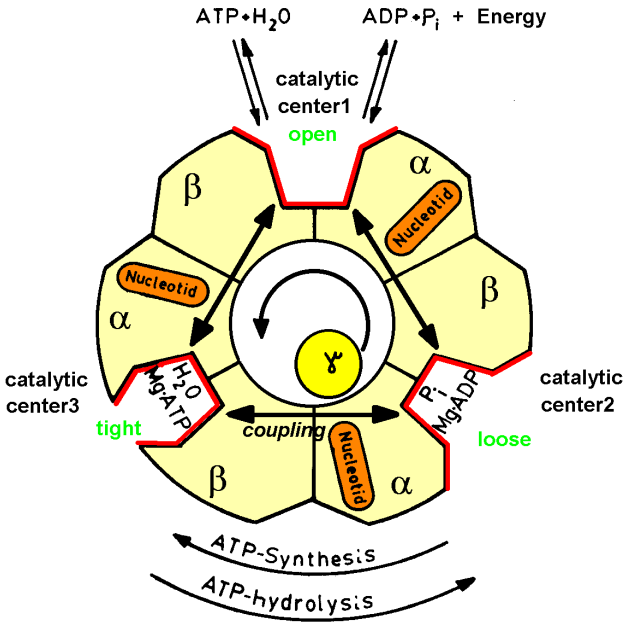

Fig.1: ATP-synthase and its catalytic fragment F_1ATPase contain a hollow assembly of six coupled large subunits $(\alpha,\beta)_3$ which bear the three catalytic and three non-catalytic sites. The coupling can be switched off by the artificial inhibitor azide.

The biological function and the regulation on the molecular level of many large proteins depends on structure dynamics of flexible domains. Those intramolecular movements are suggested indirectly by enzyme kinetics, thermodynamic studies and time resolved spectroscopic studies of enzymes, e.g. bacteriorhodopsin, photosynthetic proteins, motor proteins and ATP-synthase or its catalytic F1-fragment (F_1ATPase) [1]. In some cases structures have been estimated before and after the enzymatic catalysis by 2D-NMR or X-ray crystallography, e.g. with the proteins ras and EF-T_u using the photolysis of caged-ATP as trigger. Nevertheless the online analysis of structural dynamics during protein activity requires the unrestricted flexibility and accessibility of the protein to diffusion of substrate and products. This is in most cases only possible with protein solutions, which can be structurally investigated by time resolved small angle scattering (TR-SAXS) with high flux synchrotron radiation [2].

ATP-synthase is the major energy converter in cells of higher organisms, e.g. man [1]. It transforms energy, which is locally and transiently available at membranes of respiration or photosynthesis, to the stable transport molecule ATP. This circles in the cell and drives energy dependent processes, e.g. in muscles and enzymes.

The static structure of ATP-synthase is known from crystallography. Nevertheless the molecular mechanisms of energy conversion, the interaction in and between the three catalytic and thre non-catalytic nucleotide centers, and the regulation pathways are unknown. Obviously the molecule is a mechanical system, which can act reversibly as motor or generator driving proton transport or migration during the ATP/ADP conversion.

At the SAXS beamline of ELETTRA we have studied the structure dynamics of ATP-synthase and its catalytic headpeace F_1ATPase using the novel experimental setup for the investigation of transient structural changes of working protein solutions, which was described in the yearly SAXS-ELETTRA report 1997. In the experiments the coupling of the ATPase subunits was switched off by the inhibitor sodium azide (NaN_3). The effect and kinetics of the inhibition was studied.

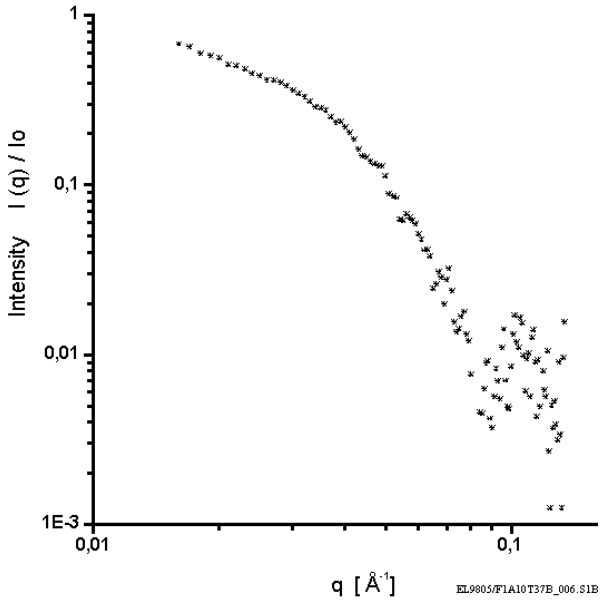

Fig.2: X-ray small angle scattering of F_1ATPase during azide inhibition obtained during a 10 s exposure. The spectrum is a frame of a structural film of 128 pictures after rapid mixing of native enzyme and azide by a stopped flow device (5g/l protein, 10mM N_3).

The samples were formed during 100 ms mixing time from enzyme solution (10g/l) and either azide stock solution or buffer in control experiments. Radiation demage was avoided by degasing and addition of 10% glycerol as radical scavenger. The temperature was held constant by a helium-jet and a remote control thermostat connected to our sample box with the quartz capillary (1 mm dia.). Under that conditions and a flux of 10^{12} ph/s at 8 keV in 0.6 x 1.5 mm spot no demage was observed during 2 h irradiation in control experiments (F_1ATPase + buffer mixture): the radius of gyration R_g = 45,12 ± 0,27 Å (in 1 min. time-frames) was constant within the error limit.

The spectra were taken by a 1D-Gas detector, which showed no reading gaps and related problems on cost of reduced yield at higher angles. During the evaluation from each picture a time dependent noise signal was subtracted from each component (see report 1997); additionaly the beam monitor B was scaled by a beam-fading signal F(t), which was obtained from the Porod-plot of the water and quartz scattering (inner standard of each time-frame; >99% in the range 0.1-0.136$Å^{-1}$) One frame of a TR-SAXS film of F_1ATPase during azide inhibition at 37°C is shown in Fig.2. By exposure during 10 s it yielded a radius of gyration of R_g = 46.25 ± 0.45 Å. The initial frames of 128 during 2h were taken logarithmically in short time (≥1s) for the detection of fast events. This single shot spectrum shows that protein structure kinetics can be observed at ELETTRA-SAXS with a time resolution down to 10 s in single shots and below 1s, if repetition is applied.

The experiments showed that the expansion of F_1ATPase and ATP-synthase found by static SAXS experiments at DESY, Hamburg [3] occurs very fast: The size increase of F_1ATPase from R_g = 45.12 ± 0.27 Å to 46.09 ± 0.26 Å (+2,15 %) by inhibition with 10 mM azide is mainly finished after <15 s (values correspond to t_i = 3 min).

During the further slow reaction with the inhibitor within 2 h, the expansion of the protein varies after 30 min (further small expansion of 1%). After >60 min a larger molecular species occurs, which is indicated by the leftmost linear range in the USAX range in Fig.3 (R_g = 71.24 ± 1.05 Å).

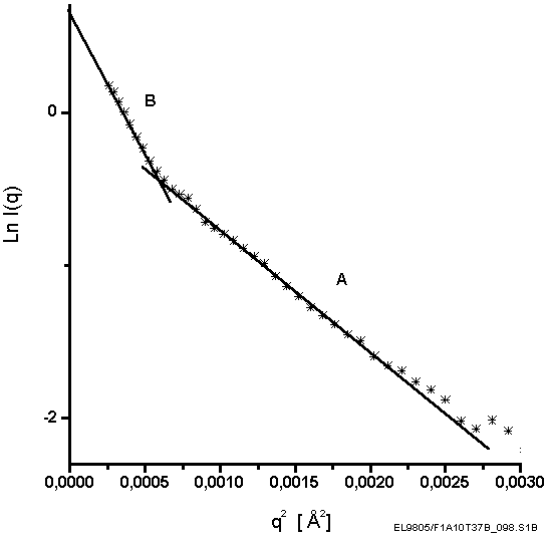

Fig.2: The Guinier representation of F_1ATPase after 90 min azide inhibition indicates the formation of a larger species (R_g = 71.24 ± 1.05 Å, Δt=1 min) by the linear range B in the USAX region (q = 0.016-0.025 $Å^{-1}$).

1. Junge, W. et al (1997) Trends Biol. Sci, 22, 420-423 and (1999) FEBS Lett. 449, 1 - 6
2. Neidhardt, A.; Nawroth, T.; Hütsch, M.; Dose, K. (1991) FEBS Lett. 280, 179 - 182
3. Nawroth, T. et al. (1997) DESY-HASYLAB report 1997, vol .I, 655 - 656