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STRUCTURAL DYNAMICS OF F\textsubscript{1}ATP\textsubscript{ase} DURING ONE REACTION CYCLE

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ATP, the universal currency of free energy in biological systems is synthesized by an enzyme complex consisting of a proton-conducting \( F_o \) unit and a catalytic \( F_1 \) moiety. Models on the reaction mechanism postulate regular conformational changes within the \( F_1 \) unit on synthesizing or hydrolizing ATP [1]. When detaching the \( F_1 \) moiety (\( F_1\text{ATP}\text{ase} \)) from the complex only the ATP hydrolyzing direction is catalyzed [2]. \( F_1\text{ATP}\text{ases} \) from different organisms all have the same subunit composition and a comparable molecular mass around 380000. There are three catalytic active sites within one molecule. It is not clear whether all three or only two of the sites contribute mainly to the ATP hydrolyzing reaction [3]. Our kinetic experiments with \( F_1\text{ATP}\text{ase} \) from Micrococcus luteus favour the two site model [4]. With two sites of the enzyme involved in hydrolysis one reaction cycle i.e. once the transition through all the hypothetical intermediate conformational states would be completed after 20 seconds at 13\(^{\circ}\)C [5]. This time range is suitable to investigate structural parameters of the transition process by time resolved X-ray small angle scattering. The X-ray scattering was performed at HASYLAB’s windowless beamline A1 with double focusing mirror using 1.3A photons. Scattered photons were counted in 1.9m distance of the sample by a position sensitive detector (\( \text{Ar}/\text{CO}_2 \) filled multi-wire proportional chamber) with a sensitive area of 290X290mm\(^2\). So a \( Q \) range between 0.018 and 0.62\text{Å}\(^{-1}\) was covered (\( Q=(4\pi\sin\theta)/\lambda, 2\theta \) being the scattering angle). The fluid sample was irradiated in a flow through quartz capillary (0.8mm inner diameter) connected to a stopped flow device. The observed count rate of scattered photons from the sample was about 10000cps on the whole detector. Data aquisition was started after triggering the enzymatic reaction by mixing with ATP (220ms mixing time) at 13\(^{\circ}\)C. From one single enzyme/substrate mixture 20 datasets in subsequent one second time intervals were generated and stored in histogramming memories as arrays of 256X256. To improve the statistical error the time resolved scattering
data from 5 F$_1$ATPase/ATP mixtures were accumulated and reduced to one-dimensional scattering profiles by radial averaging. Fig.1 shows the dynamics of the molecular shape of the F$_1$ATPase occuring during one reaction cycle. The radius of gyration calculated from the innermost part of the scattering spectra increases after 9 seconds from starting the enzymatic reaction to 46.6±0.7Å. This expanded state in the middle of the reaction cycle is kept for 4 seconds and clearly deviates from the radius of gyration (43.8±0.5Å) found at the beginning and the end of the reaction cycle.

Fig. 1: Dynamics of the molecular expansion of F$_1$ATPase detected during the first reaction cycle of the hydrolytic catalysis.

Such a large change in the molecular structure could be due to a subunit rearrangement during catalysis. So the F$_1$ molecule passes at least two structural distinguishable intermediate states upon hydrolizing ATP (Fig.2) were the R- and S-phase are probably not identical.

Fig. 2: F$_1$ATPase passes possibly three intermediate conformations during one (100%) reaction cycle of ATP cleavage: Starting (0%) with a compact molecular shape (S-phase) the enzyme passes a short expanded E-phase (45–65%) and returns to the initial state through a further compact conformation (R-phase).